ETHICS & MEDICS
OCTOBER 2020 VOLUME 45, NUMBER 10
A Commentary of The National Catholic Bioethics Center on Health Care and the Life Sciences

GAPS IN EMBRYO MODEL ETHICS

Kevin Wilger

This past January, the International Society for Stem Cell Research (ISSCR) announced that it will be updating its *Guidelines for Stem Cell Research and Clinical Translation* to include advances in the field of human embryo modelling.[1] Pioneers initially stated that because they do not utilize conventionally fertilized human embryos, embryo models could potentially circumvent many concerns regarding human embryonic research.[2] However, more recent publications have admitted to some ethical challenges regarding the technology.[3] Thus, the ISSCR cautions in its announcement that these embryo models do in fact raise "important scientific, clinical, ethical, and societal issues for researchers, regulators, and funding agencies."[4] The following essay reviews the science, ethical debates, and guidelines for the responsible progression of embryo modelling.

Embryos and Embryo Models

Embryo models, sometimes called synthetic embryos, are attempts to recreate embryo development with pluripotent stem cells rather than embryos. Goals of this field of research include knowledge of early human development and prevention of adverse events in pregnancy (such as failure to implant, miscarriage, genetic and developmental defects, and so on). The field is relatively young; thus, the types of models and the methods used will vary depending on the investigator and the stage of embryonic development that is being studied.

For example, one type of embryo model is called the blastoid, and it attempts to mimic the blastocyst. The blastocyst is characterized by a sphere of cells called the trophectoderm (TE), a fluid-filled cavity (blastocoel), and an inner cell mass (ICM).[5] In research the blastocyst can be harvested via in vitro fertilization and developed for culture in media. In contrast, the blastoid is created by separately culturing the TE and ICM cells, placing TE cells over ICM cell aggregates in a specially designed microwell plate, and then adding molecules in culture that stimulate blastocoel formation. After two-and-a-half days, those TE and ICM aggregates that went on to form a blastocoel have expanded and stabilized. If the assembly is the right size (70–110 um), is circular, and has a single TE layer encapsulating a cavity and ICM, then it is deemed to be a blastoid and available for analysis.[6] The blastoid is potentially a powerful research tool because of scientists' ability to genetically modify the TE and ICM cells separately while studying their separate effects on blastocyst development.

Investigators use another type of embryo model, called the gastruloid, to study the development of the mammalian body from blastocyst to multilayered gastrula, known as gastrulation.[7] In gastrulation the blastocyst begins to develop axes of the body and three cell lineages: the ectoderm, endoderm, and mesoderm. The gastruloid is a three-dimensional, multicellular aggregate of embryonic stem cells that has the ability to develop three orthogonal axes and the three cell lines of the gastrula.[8] Much of the work surrounding gastruloids has been done with mouse models, but in June of this year, *Nature* published the first use of human gastruloids to study anteroposterior development of the embryo, and another article in preprint builds from this model to study early human spinal cord development.[9]

Other constructs discussed by the ISSCR include the ETX embryo model, the amniotic-epiblast model, and two-dimensional micropatterned stem cells. In general, embryo models utilize the self-organization of stem cells to model aspects of development. An increase in human studies and promising results has triggered investigators to raise ethical questions regarding their research.

Call for Debate

In a December 2018 *Nature* commentary, several members of the ISSCR called for rigorous international debate on the topic.[10] Since their call for debate, and at the time of writing, a Google Scholar search revealed that the commentary has been cited over twenty times, but only a handful of those citations directly address the ethical issues identified by the original authors.

One of those citations is my own essay published in the autumn 2019 issue of the *National Catholic Bioethics Quarterly*.[11] There I try to establish uncontroversial ethical common ground with the authors and address the questions and recommendations of the commentary given that common ground. To quickly describe that common ground, human beings are organisms, and consequently, there are objects that are naturally good for them (e.g., food, water, and love) and help them thrive and flourish. Conversely, some objects are naturally bad (e.g., starvation, dehydration, and hate) and lead to their demise. Some objects can always be bad for humans, like rape or destruction, regardless of the age or size of the human. Willing or intending these bad objects toward another human is *morally bad*. Human embryos are humans; therefore, experimenting and destroying human embryos is *bad for them*, and willing or intending their destruction is morally bad.

The crucial factor is whether the embryo models are equivalent to human embryos. If they are equivalent to human embryos, researchers should not experiment on or destroy them. Conversely,

if the model is demonstrably not a human organism, research could *potentially* move forward. If researchers are unsure given the available data, I argue that caution is necessary and that experiments on human models should not move forward to collect more data.

Organismic Potential

With that background in mind, we now turn to Insoo Hyun and colleagues' February 2020 article in *Stem Cell Reports*, "Toward Guidelines for Research on Human Embryo Models Formed from Stem Cells,"[12] which proposes recommendations for the updated research guidelines to come in 2021. Prior to addressing the specific recommendations, the phrase *organismic potential* (or correlates) must be addressed, as it appears throughout the report in several instances and is used as a criterion for oversight. For example, the authors' second recommendation for oversight states, "Culture systems that do not model the integration of all embryonic and extraembryonic lineages or models that clearly lack the potential to form a full organism are exempt from mandatory review" (173). Since this criterion is so essential to the authors, additional scrutiny should be given to the proper understanding of organismic potential and its erroneous uses.

Organismic potential first appears in the guidelines in relation to whether embryo models fall under any of several legal definitions of human embryos. For example, Australia's legal definition of the human embryo includes the potential to develop the primitive streak of the embryo, whereas Japan's definition indicates a potential to develop in utero (human or animal). To make matters more confusing, the section in the current ISSCR guidelines on prohibited research activities seems to be defining human organismal potential in embryo models as a potential for something temporally beyond the activity that is already occurring in embryo models: "*In vitro* culture of… organized embryo-like cellular structure with human organismal potential, regardless of derivation method, beyond 14 days or formation of the primitive streak, whichever occurs first" (173). Considering these definitions, Hyun and colleagues note uncertainty in the definition of organismic potential.

Two test cases are offered to narrow the search for a unifying definition. First, Hyun and colleagues' emphasis on a model's "potential to form a full organism" *should not* indicate that the entity is an organism if and only if it is able to become the *mature* version of itself with all its powers and faculties. This is much too high of a bar to determine the presence of a whole human organism, as it would exclude those infants which tragically perish prior to adulthood. The embryo model could be a human organism that because of disease or design, dies prior to maturation.

Second, this phrase should not indicate that the entity is an organism if and only if it has all of its typical parts. Consider a genetic disease or malformation which leads to an infant's being born without critical organs or extremities; the child is still a human organism, not one with potential to be a human. If an embryo model were to be manufactured missing a part found in a typical embryo, it does not necessarily follow that the model is not a human organism or human embryo.

The Scholastic intellectual tradition has much to offer to the question of organismal potential, in particular the recognition of the realities of *substantial forms*, *ends (teleology)*, and *properties*.[13] The substantial form, or substance, is the intrinsic principle of activity in a natural object, which inclines its matter toward a number of goals or ends. The substantial form of a tree directs its activity toward the ends of nutrition and growth by absorbing water and generating sugars from sunlight. Given that nature is composed of substances with activity toward ends, there are bound to be a number of observable characteristics, or properties, that *typically* manifest in nature. For a substance, these properties cannot be reduced to the activity of its parts in isolation or in aggregate.[14]

It is here where this understanding of *being* aligns with the classical biological properties of life and organism. If the entity (the entire model) is observed to have properties like cellular organization, ordered complexity, sensitivity, metabolism, development, regulation, and homeostasis, then it may be *here and now* a distinct human organism, not a potential one.[15] These criteria are drastically different from the ability to become fully mature or the presence of the entirety of parts; moreover, these criteria are more cohesive with the aforementioned test cases of the child that perishes before adulthood and the infant born with missing organs. Throughout their lifetime, these hypothetical children have organization, ordered complexity, sensitivity, and so on; so they are correctly thought to be members of the human species. Only when disease or accident attacks their bodily integrity do they perish and cease to be organisms.

The following is a short survey of data related to embryo models mentioned by Hyun and colleagues.

1. The blastoid (1) has embryonic and trophoblast stem cells that form blastocyst-like structures in vitro, (2) can form "primitive endoderm-like" cells, (3) has the cell count of a mid-stage blastocyst, and (4) appears to induce changes in the mucosal lining of the mother's uterus when implanted.[16]
2. Similarly, the ETX embryo model (1) forms the blastocyst-like structures and primitive endoderm-like cells, (2) forms embryonic-abembryonic axes, and (3) begins the postimplantation morphological transition in vitro.[17]
3. As noted above, the gastruloid model has (1) axial elongation of induced pluripotent stem cell aggregates (up to 1 mm) over 168 hours, (2) the three orthogonal axes that serve as a reference for the organization of the derivatives of the three germ layers, and (3) gene expression that "mimics" an embryo that is 6.5 to 9.5 days old.[18]

Based on this synopsis, the data seem to point to organismal activity in each model, which is not surprising *given that this is the aim of the embryo model project in the first place*. The cellular structure is morphologically similar to mouse embryo analogs, indicating a cellular organization and ordered complexity. Growth in the overall structure, transition of cell types, and inducement of uterine modifications all suggest sensitivity, development, and metabolism in the embryo model. At the very least, the data above do not support the contrary view that these models are non-intact.

The human data have not progressed as far as the mouse data. Again, from the assessment in the respective publications:

1. In the epiblast-amniotic model, human pluripotent stem cells display (1) epiblast-like lumenogenesis, (2) formation of a bipolar embryonic sac, (3) germ cells and primitive streak cells, and (4) gastrulation initiation.[19]
2. Geometrically constrained, two-dimensional patterns of human embryonic stem cells form "an outer trophectoderm-like ring,

an inner ectodermal circle and a ring of mesendoderm expressing primitive-streak markers in between."[20]

3. Human gastruloid models form axes and the three germ layers as well as anteroposterior elongation.[21] One study, currently in review, used human pluripotent stem cells to model neural tube extension, which resulted in morphology and gene expression consistent with neural tube development.[22]

As in the mouse models, correlates of the development, organization, and complex ordering of human embryos seem to also exist in these models. The question of whether parts of the human, rather than a whole human, are being modelled is a difficult one because the development stages modelled are the ones where "parts" are just starting to take form. Again, the results suggest that organisms are being formed from stem cells more than they suggest that parts of a human or a second entity is being generated.

One anticipated criticism of the approach described above is that the criteria for organism overcorrect the errors of the ISSCR and set too low a bar for what is considered a bona fide human being. Granted, with more space, the other hard cases (such as hydatidiform moles, parthenotes, and activated oocyte cytoplasts) need to be parsed out. However, given the gravity and epistemic difficulty of the question being asked, it is more prudent to take a position of principled caution and err on the side of including more organisms as bona fide human beings than to adopt an approach that chances destroying humans.

Questions regarding Proposed Ethical Considerations

We can now turn to the ethical considerations that will seemingly inform the proposed guidelines. Hyun and colleagues comment on six ethical considerations regarding (1) partial conceptus or limited development models, (2) entire conceptus and integrated development models, (3) current oversight of human embryo research, (4) funding, (5) intent of the research, and (6) the potential benefit and quality of proposed experiments. Below, I offer four initial questions regarding the most glaring concerns from these considerations.

What Is the Proper Role of Animal Data regarding Embryonic Model Development?

In the third section of their article, Hyun and colleagues provide warning about generalizing conclusions from the development of an embryo model of a different species to the development of human embryo models. Specifically, the authors question whether the absence of "developmental competence," or the ability to mature, in animal models indicates the same in human models. Here the authors are commenting on a hypothetical experiment to determine the organismic potential of certain embryo models. They indicate that such experiments would seemingly violate prohibitions on research activities that involve culturing past fourteen days (or the existence of the primitive streak) and ex utero or animal-uterus gestation. The point is that performing similar experiments with animal embryo models may be of no use in alleviating this problem, because of their lack of generalizability to the human model.

However, fast-forward to the first ethical consideration, and we see that animal data are in fact being used to guide permissive recommendations regarding the partial conceptus because animal models indicate that gastruloids and other constructs cannot develop into embryos in the absence of extra-embryonic tissue. In the second ethical consideration, the authors again draw from mice studies to inform caution about embryo models that attempt to model the entire conceptus, because mouse models indicate that blastoids might develop if implanted in utero.

This reasoning from these two examples seems to contradict the logic from the previous section. Which applies: Is there a relevant species difference that invalidates generalizing data from mice to human? Or are the findings from mouse studies valuable representations of human model development that can help guide ethical considerations? Perhaps the authors were indicating that the species difference is relevant only to generalizing the "organismal developmental" results from a mouse model, but that other measures are generalizable. However, as the text stands, it is unclear how the authors view the role of animal data.

Which Approach to Regulation of Embryo Research?

The third ethical consideration states that regulation for synthetic embryos that "model the integrated development of the entire conceptus should be informed by the *current approach to regulation of embryo research*" (172, emphasis added). Of course, if this statement is true, which of the current approaches should be taken? Presumably, the authors prefer the permissive regulatory environments of the nations they highlight: the United States, the United Kingdom, Canada, and Australia. They do not mention nations where human embryo research is restricted, such as Italy and Germany.

The neutral tone and lack of explicit indication leave the reader with the following impression: investigators of integrated embryo models should follow the current human embryo laws of their jurisdiction. However, the ISSCR is in the business of policy and advocacy not neutrality. Even Hyun herself called on regulators to revisit the standard fourteen-day rule of human embryo research.[23] The ISSCR policy positions state that it "support[s] all forms of stem cell research, performed under rigorous and transparent oversight."[24] For transparency and clarity, this recommendation should be revised to explicitly indicate the preferred regulatory approach of the ISSCR.

Which Intentions Are Relevant to Regulation and Why?

The fifth ethical consideration indicates that regulation should "take into account the intent of the research" (173). For example, synthetic embryos should not be used to produce a pregnancy, but they should be used to model improvements to reproduction. This is the totality of ethical consideration five, and as such, the authors have left the policy makers without any *reason* why one intention should be prohibited and the other should be exempt. What is the ethical reasoning behind this recommendation? Presumably Hyun and colleagues are concerned with potentially lasting modifications to the human germline in the reproductive application, which are not present in modelling experiments, but a thorough discussion of permissible and impermissible intentions is needed.

What Ethical Benefits?

The last ethical consideration emphasizes that regulators and reviewers should consider the "ethical and practical benefits of replacing human embryo material with broadly available stem cell lines" (173). Is this not placing the cart before the horse? To revisit,

THE NATIONAL CATHOLIC BIOETHICS CENTER

6399 Drexel Road, Philadelphia, PA 19151–2511 www.ncbcenter.org

ETHICS & MEDICS
VOLUME 45, NUMBER 10
OCTOBER 2020

it has not been shown that these entities are formally distinct from human embryos, and obviously this is the source of much of the ethical debate surrounding so-called synthetic embryos. We do not know whether there will be any ethical benefits until the status of the synthetic embryo is known.

As before, I applaud Hyun and colleagues for proactively and transparently calling for a debate on this field of research. I hope that their report generates more rigorous public discussion on synthetic embryos, especially among Catholics. Moving forward, the conversation should focus on the philosophic and scientific determination of organisms. This should be animated by a principle of caution because collecting evidence on the properties of embryo models puts them in danger of destruction; and if the synthetic human embryo is in fact a human embryo, then it deserves the respect afforded to all humans.

Kevin Wilger, MS, is a research engineer for Cook Research Incorporated and a catechist at the Cathedral of Saint Mary of the Immaculate Conception in Lafayette, Indiana. The views expressed in this essay are the author's alone and do not necessarily reflect the views of his employer.

Notes

1. International Society for Stem Cell Research, "ISSCR Statement on Ethical Standards for Stem Cell–Based Embryo Models," news release, January 16, 2020, https://www.isscr.org/news-publicationsss/isscr-news-articles/article-listing/2020/01/16/isscr-statement-on-ethical-standards-for-stem-cell-based-embryo-models.
2. Institute of Molecular Biotechnology, "Welcome to the Rivron Lab," accessed September 29, 2020, https://www.imba.oeaw.ac.at/research/nicolas-rivron/. The Rivron Lab refers to its models as *blastoids*: "Because blastoids are formed solely from stem cells, their use in research avoids ethical concerns surrounding the use of blastocysts. … Blastoids are not considered to be a legal equivalent of blastocysts."
3. Laboratory for Synthetic Development, "The Ethic of Stem Cell–Based Embryo Models," accessed September 29, 2020, https://www.blastoid.org/the-ethics-of-synthetic-embryos.
4. ISCCR, "Ethical Standards for Stem Cell–Based Embryo Models."
5. Keith L. Moore, T.V.N. Persaud, and Mark G. Torchia, *The Developing Human: Clinically Oriented Embryology* (Philadelphia: Elsevier, 2015), 33, ProQuest Ebook Central.
6. Nicolas C. Rivron et al., "Blastocyst-Like Structures Generated Solely from Stem Cells," *Nature* 557.7703 (May 2, 2018): 106–111, doi: 10.1038/s41586-018-0051-0.
7. Moore et al., *Developing Human*, 51–52.
8. Leonardo Beccari et al., "Multi-Axial Self-Organization Properties of Mouse Embryonic Stem Cells into Gastruloids," *Nature* 562.7726 (October 3, 2018): 272–276, doi: 10.1038/s41586-018-0578-0.
9. Naomi Moris et al., "An in Vitro Model of Early Anteroposterior Organization during Human Development," *Nature* 582.7812 (June 11, 2020): 410–416, doi: 10.1038/s41586-020-2383-9; and A.R.G. Libby et al., "Axial Elongation of Caudalized Human Pluripotent Stem Cell Organoids Mimics Neural Tube Development," preprint, posted March 6, 2020, doi: 10.1101/2020.03.05.979732.
10. Nicolas Rivron et al., "Debate Ethics of Embryo Models from Stem Cells," *Nature* 564.7735 (December 13, 2018): 183, doi: 10.1038/d41586-018-07663-9.
11. Kevin Wilger, "Embryo Models Derived from Stem Cells: A Response to Nicolas Rivron and Colleagues," *National Catholic Bioethics Quarterly* 19.3 (Autumn 2019): 391–399, doi: 10.5840/ncbq201919329.
12. Insoo Hyun et al., "Toward Guidelines for Research on Human Embryo Models Formed from Stem Cells," *Stem Cell Reports* 14.2 (February 2020): 169–174, doi: doi: 10.1016/j.stemcr.2019.12.008. All subsequent citations appear in the text.
13. Edward Feser, *Scholastic Metaphysics: A Contemporary Introduction* (Neunkirchen-Seelscheid, DE: Editiones Scholasticae, 2014), 160–164, 230–234.
14. For a discussion of parts versus whole, see Samuel B. Condic, and Maureen L. Condic, *Human Embryos, Human Beings: A Scientific and Philosophical Approach*, (Washington, DC: Catholic University of America Press, 2018), chap. 8.
15. Peter Raven et al., *Biology*, 8th ed. (New York: McGraw-Hill, 2007), 504; and David Sadava et al., *Life: The Science of Biology*, 8th ed. (London: MacMillan, 2008), 4–9.
16. Rivron et al., "Blastocyst-Like Structures," 106–111.
17. Berna Sozen et al., "Self-Organization of Mouse Stem Cells into an Extended Potential Blastoid," *Developmental Cell* 51.6 (December 16, 2019): 698–712, doi: 10.1016/j.devcel.2019.11.014.
18. Beccari et al., "Multi-Axial Self-Organization Properties," 272–276.
19. Yi Zheng et al., "Controlled Modelling of Human Epiblast and Amnion Development Using Stem Cells," *Nature* 573.7774 (September 11, 2019): 421, doi: 10.1038/s41586-019-1535-2.
20. Aryeh Warmflash et al., "A Method to Recapitulate Early Embryonic Spatial Patterning in Human Embryonic Stem Cells," *Nature Methods* 1.8 (August 2014): 847, doi: 10.1038/nmeth.3016.
21. Moris et al., "Model of Early Anteroposterior Organization," 410–416.
22. Libby et al., "Caudalized Human Pluripotent Stem Cell Organoids."
23. Insoo Hyun, Amy Wilkerson, and Josephine Johnston, "Embryology Policy: Revisit the 14-Day Rule," *Nature* 533.7602 (May 12, 2016): 169–171, doi: 10.1038/533169a.
24. International Society for Stem Cell Research, "Positions and Statements," accessed June 2, 2020, https://www.isscr.org/policy/positions-and-statements.

Ethics & Medics is a publication of The National Catholic Bioethics Center. Regular annual subscription rates for twelve issues include both the print version by mail and online access at www.ncbcenter.org/em: United States, $28; foreign $38; institutional $55. Individual copies are available for $3 each. To subscribe, please write to The National Catholic Bioethics Center, PO Box 596, Wynnewood, PA 19096, e-mail orders@ncbcenter.org, or phone (215) 877–2660. Publisher: Joseph Meaney, PhD. Editor: Edward J. Furton, MA, PhD. ISSN 1071–3778 (print), ISSN 1938–1638 (online). To submit an essay or request submission guidelines, please e-mail submissions@ncbcenter.org.