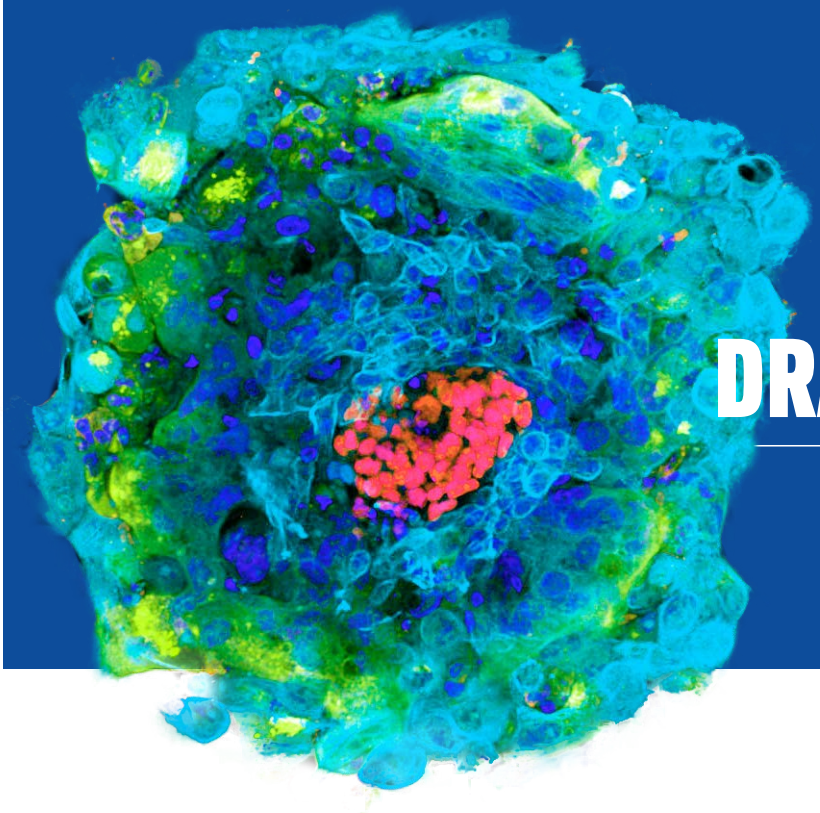
center for
HEALTH + BIOSCIENCES
Rice University's Baker Institute for Public Policy



DRAWING THE LINE

ETHICAL, POLICY, AND SCIENTIFIC PERSPECTIVES ON U.S. EMBRYO RESEARCH

CELL-CULTURE MODELS OF EARLY HUMAN DEVELOPMENT: SCIENCE, ETHICS, AND POLICY

Kirstin R.W. Matthews, Ph.D.
Jason Scott Robert, Ph.D.
Ana S. Iltis, Ph.D.
Inmaculada de Melo-Martin, Ph.D.
Daniel S. Wagner, Ph.D.

February 2019

Acknowledgments

This report is part of a project "Drawing the Line: Assessing and Analyzing the U.S. Rule on Embryo Research from Ethical, Political, and Scientific Perspectives," which is funded by The Greenwall Foundation. The assessment is part of a series of publications available online at https://www.bakerinstitute.org/research/ethical-policy-and-scientific-perspectives-us-embryo-research/. The authors would like to thank the Rice University staff and students who helped research and edit the manuscript, including Sharon Tsao and Sophia Huang.

Kirstin R.W. Matthews, Ph.D.
Jason Scott Robert, Ph.D.
Ana S. Iltis, Ph.D.
Inmaculada de Melo-Martin, Ph.D.
Daniel S. Wagner, Ph.D.
"Cell-Culture Models of Early Human Development: Science, Ethics, and Policy"

Technical improvements in developmental biology create new opportunities for research but also new ethical and regulatory challenges. In the early 20th century, human embryology research was initiated with embryos obtained by physicians after obstetrical surgery (such as an emergency hysterectomy) or pregnancy disruption (including miscarriage in the first eight weeks of pregnancy). Embryo specimens were preserved, fixed, and often sectioned or stained for analysis, sometimes without the knowledge of the female patient, and studied for decades (Morgan 2004). With the advent of *in vitro* fertilization (IVF) techniques that allowed the creation of embryos outside of a woman's body in the early 1970s, scientists and clinicians faced the prospect of not only helping those experiencing infertility to have children, but also of studying any remaining embryos not transferred to a woman's uterus. These so-called leftover embryos afforded the opportunity to study living human embryo specimens instead of just dead ones.

Instead of studying human embryos directly, scientists are now deriving novel, laboratory-grown entities from human hESCs or iPS cells.

As a function of the political and cultural sensitivity of human embryo research, several jurisdictions instituted a rule or guideline stipulating the impermissibility of research on human embryos beyond 14 days post fertilization (dpf), although the United States was not one of these jurisdictions.[1] At the time, *in vitro* embryos could not be kept alive for more than a few dpf. Thus, this limit, first suggested in the 1970s, has remained out of reach technically until now. In 2016, scientists developed techniques to nurture the survival of human embryos *in vitro*, enabling embryo research up to and potentially beyond 14 dpf. Ultimately, the scientists destroyed the samples when they reached day 14 (Shahbazi 2016; Deglincerti 2016a).

In recent years, several laboratories have made significant strides toward studying human embryogenesis without destroying human embryos in the process. Instead of studying human embryos directly, scientists are now deriving novel, laboratory-grown entities from human embryonic stem cells (hESCs) or from induced pluripotent stem (iPS) cells[2] (Warmflash et al. 2014; Van den Brink et al. 2014; Deglincerti et al. 2016b). These entities could permit studies of early human development like those already permissible with human embryos, as well as studies of human embryonic development stages that are currently off-limits with human embryos. For example, the phenomenon of gastrulation, which usually starts to take place around 15 dpf, could be studied in these entities.

[1] The United States does not have a formal policy limiting human embryo to 14 dpf; therefore, scientists are not restricted on their research. However, the federal government does not fund any research on human embryos (where they can be destroyed or subjected to risk of harm) via the Dickey-Wicker Amendment. The ban does not impact private or state funding of research (Matthews and Yang 2019).

[2] iPS cells are created by reprogramming specialized or differentiated cells (such as a skin or blood cell) to function similar to ESCs. This method allows researchers to create patient-specific cells that are easy to grow *in vitro*. These cells can be differentiated in the lab to examine how specific patient genotypes affect disease development or progression.

Currently, these entities are relatively simple proxies for the human embryo. With them, scientists seek to replicate discrete aspects of human embryo development in culture in order to examine developmental processes in greater detail in controlled and reproducible experiments. However, because of their simplicity, these entities do not eliminate the need for human embryo research. Thus, researchers are interested in creating more complex entities that would replicate human embryo development in greater detail. This would allow scientists to capture the complex dynamics and integrated signaling networks that are required for human development.

These novel entities that replicate human embryo development are controversial in several ways. First, insofar as their development requires hESCs, their creation is objectionable to those who reject embryo destruction for research purposes. Second, given that they are ersatz embryos, there are questions about whether they are sufficiently human-embryo-like to give accurate information about human development. Third, because they are artificial entities, it is not clear whether they warrant ethical and regulatory oversight as if they are human embryos. That of course does not mean that no regulatory oversight would be appropriate, but the issue is whether research with these entities should be subject to separate ethical and regulatory oversight. Fourth, there are important disagreements about what these novel entities should be called. The terminology is important, not least because referring to them as embryos or embryo-like, for instance, triggers a variety of responses that may or may not be appropriate given the nature of these entities (Baylis and Krahn 2009).

In this paper, we delve into these novel entities by briefly reviewing their biological character and the processes by which they are being or may be created. We then turn to the question of nomenclature. Next, we explore how these entities fit or fail to fit into existing regulatory schemes and research policies, and raise and address ethical questions about these entities. Finally, we make a recommendation about how research with them should be governed.

What Are These Entities?

Embryonic stem cells (ESCs) have been derived from several mammals including mice, monkeys, and humans. These cells are similar to the cells of the epiblast in the early development in these animals. They can give rise to all cells of the embryo in the case of mice and primates. While the full potential of human cells is unknown due to ethical constraints on human embryo research, hESCs can become many different cell types in culture, depending on the culture conditions. Recent efforts have attempted to recapitulate early development by culturing mouse ESCs with cells that represent extraembryonic cell types. In the case of human cells, novel embryo-like entities can be derived in several ways, including the use of micropatterned colonies of hESCs cultured with several regulatory factors that induce pattern formation in the embryo. One example of such an entity is a "gastruloid," a simple form of self-organized hESC colonies of controlled size and shape with identifiable features, such as a primitive streak (usually found in human embryos only at or around 14 dpf) and ordered layers of cells from all three germ layers (which usually happens around 15 dpf) (Aach et al. 2017; Deglincerti et al. 2016b; Van den Brink et al. 2014; Warmflash 2014).

Of note, all of these human-cell-derived entities are poorly organized relative to intact embryos, and they lack extraembryonic and maternal cell types important for robust and accurate embryonic development. Nevertheless, the potential for future experiments that accurately mimic human embryos means we should consider the ethical issues they may raise before they are a reality.

While research with these entities is still rudimentary, their use has led to some successes. They have the advantage of reproducibility, since hundreds of them can be made with exactly the same genotype. They are also highly manipulable, as their cells may be engineered to mutate specific genes or add genes that allow for direct detailed observation of living cells. Yet it is difficult to know how closely these gastruloids resemble human embryos undergoing or having undergone gastrulation without comparing them to actual developing human embryos (which is impermissible in some jurisdictions).

Given sufficient advances in cell culture methods, these novel entities may ultimately be 'made to order' at any stage of embryonic development.

Given sufficient advances in cell culture methods, these novel entities may ultimately be 'made to order' at any stage of embryonic development. They could potentially mimic both earlier and later stages of embryo development (such as the blastocyst stage, which normally occurs around 3 to 6 dpf, or the neurula stage, which normally occurs around 20 dpf). As with simpler entities, determining whether these more complex ones are adequate will require comparative research with actual human embryos. The creation of embryo models of a more advanced age leads researchers into ethically risky territory. For instance, kidney organoids are *in vitro* cultured cells, typically ESCs, that are organized similar to a kidney. However, a recent study revealed unexpected muscle and brain cells in kidney organoids (Wu et al. 2018), highlighting the need for careful scientific and ethical analysis of these entities.

What Should We Call Them?

Scientists have offered several names for these novel entities, including "embryoids," "embryoid bodies," "synthetic embryos," "synthetic human entities with embryo-like features (SHEEFs)," and "micropatterned hESC colonies" (Warmflash et al. 2014; Van den Brink et al. 2014; Simunovic and Brivanlou 2017; Aach et al. 2017). Of note, several of these names include reference to the embryo. Furthermore, two of them refer to the synthetic origins of these entities. In so doing, both of these terms may lead to political and ethical controversy. For instance, synthetic entities could summon public fears of artificial biology. Similarly, referring to these entities as "embryos" raises the prospect of regulating their use according to existing human embryo research policies, even if they are created at a certain developmental stage without any previous development as an embryo.

Because of these issues, some scientists refer to these novel entities in ways that avoid references to their origins or similarities with embryos. For example, the original paper referred to these entities as "micropatterned hESC colonies," which describes their origins but not their similarities to embryos in their current state (Warmflash et al. 2014). Others have tried to be more explicit by calling attention to the specific developmental stage they are meant to mimic. For instance, entities that approximate the formation of gastrulation are referred to as "gastruloids" (Van den Brink et al. 2014) and those approximating the formation of the blastocyst as "blastoids" (Rivron et al. 2018). Of course, these choices are not without criticism. The apparent descriptiveness of the terms deflects attention from the resemblance these entities have to embryos.

Although hCCMED might not be as memorable, we believe it has the advantage of being informatively descriptive.

We propose referring to these entities collectively as "human cell-culture models of early development" (hCCMEDs). Although hCCMED might not be as memorable as some of those mentioned earlier, we believe it has the advantage of being informatively descriptive. While this terminology conceals the similarities these entities have with embryos, we believe such concealment serves to focus attention on morally relevant issues without engaging in contentious debates. Wherever we consider it useful to describe a particular kind of hCCMED in a more specific way, we do so. For instance, we refer to an entity that approximates gastrulation as a "human cell-culture model of gastrulation."

Ethical Issues Raised by hCCMEDs

Aside from the issue of nomenclature, hCCMEDs raise ethical challenges concerning their moral status. Are hCCMEDs sufficiently human-embryo-like to be treated morally as if they were human embryos? Of course, even if the answer to this question is “no,” hCCMEDs may still be thought as having moral status or deserving respect or consideration, which would have implications for how they ought to be treated. This answer also impact how research using hCCMEDs are regulated, if at all.

The Moral Status of hCCMEDs

There are several grounds that have been advanced as necessary and/or sufficient for human embryos to possess full or partial moral status: species membership, individuality, some physical characteristics, cognitive capacities, and potentiality. Below we consider each of these in turn in regard to hCCMEDs (see also Hyun et al. 2016; Aach et al. 2017; Harvard University 2018).

Species membership: Whether hCCMEDs belong to the human species depends on whether they are more like human organs or organoids (which are just a part of a human,

and not full members of *Homo sapiens*) or more like human embryos (which are). In some respects, an hCCMED is more similar to a kidney organoid or a human cell-culture model of the kidney, than to a human embryo. Both the hCCMED and the kidney organoid enable the study of some key features of human development, structural and functional anatomy, and physiology that may otherwise be technically impossible to study or scientifically more difficult (or less tractable) via other means. It is true that an hCCMED is meant to mimic certain aspects of the human embryo and thus it might be thought of as akin to human embryos. Nonetheless, its status as a model of embryonic development suggests that it is not sufficiently embryo-like to count as a member of *H. sapiens*. If future research with hCCMEDs turns out to produce entities with even more similarities to human embryos, judgments about whether they belong to the human species could change.

Individuality: Insofar as something akin to "twinning" can be controlled in the research setting, hCCMEDs are biological individuals only at the will of scientists. The hESCs that would likely be used to create hCCMEDs can essentially be expanded indefinitely in culture. Creating many identical hCCMEDs is likely because experimental reproducibility is an essential advantage of using hCCMEDs. Given that this conditional criterion can and likely will be violated easily, it is unlikely to be used as a criterion to evaluate the moral status of hCCMEDs.

Physical characteristics: The physical characteristics of hCCMEDs raise the most compelling grounds for moral status considerations (Aach et al. 2017) because they are lab-created entities that resemble human embryos in their shape, size, and identity of their component cells. Should hCCMEDs develop neural tissues, for instance, some might argue that these tissues could subserve the emergence of morally relevant traits (such as sentience, discussed below), and that the physical nature of these tissues and the capacities they eventually enable are sufficient for moral status. But insofar as hCCMEDs are created for research purposes exclusively and not as part of a reproductive enterprise, the likelihood that hCCMEDs will develop into physically and physiologically complex enough entities to be ersatz embryos is negligible. Even so, this research warrants critical scrutiny to ensure this prediction remains accurate.

Cognitive capacities: Like human embryos, hCCMEDs also lack sentience or consciousness. Nonetheless, possible advances in long-term culture conditions may allow hCCMEDs to develop for months and reach the equivalent fetal stages of human development where sentience exists. Given that many would take sentience to be a characteristic that confers at least partial moral status, consideration should be given to limiting the time of culture or developmental stage as technology progresses.

Potentiality: The potential for an hCCMED to become a person is virtually nonexistent without significant advances in *in vitro* culture conditions that could allow an hCCMED to implant in a woman's uterus. This is not possible at this point, but technical developments might allow scientists to create entities with such potentiality and an artificial uterus to maintain an embryo *ex vivo*. To the extent that potentiality confers any degree of moral

status, hCCMEDs that have the potential to become persons would possess such degree of moral status. Nonetheless, even though hCCMEDs lack the ability to implant at this point, it may prove useful to think of them as possessing potentiality in the same way as many commentators think of leftover human embryos destined for destruction rather than for reproductive purposes. An hCCMED's developmental potential, short of personhood, rests firmly in the hands of the scientists culturing it, and in those of any governance body overseeing the research.

Implications of Claims About the Moral Status of hCCMEDs on the 14-Day Guideline

To have moral status is "to be morally considerable, or to have moral standing." This means our "needs have moral importance in their own right" (Warren 1997, 3). Further, others have or can have obligations to us, and there are limits on how they may treat us. For hCCMEDs, as for human embryos, having moral status does not by itself determine how they should be treated, just that they should be given consideration in moral deliberations (Warren 1997). Accordingly, judgments about whether hCCMEDs have moral status at all, or the degree of moral status they might have, are insufficient to resolve questions about the moral permissibility of their creation and disposition. Whatever the answer about the moral status of hCCMEDs or lack thereof, it is worthwhile to briefly assess how the existence of these entities could impact deliberations about the 14-day guideline governing human embryo research.

If hCCMEDs are deemed to have full moral status, then hCCMED-destructive research may be morally impermissible insofar as society would have a strong warrant to protect them. As in the case of human embryos, however, many might hold that such warrant may be overridden by other moral considerations in very limited circumstances. If, on the other hand, hCCMED characteristics confer a lesser degree of moral status, research on them could be morally permissible under some circumstances. For instance, hCCMED-destructive research could dramatically reduce the scientific need for embryo-destructive research beyond 14 dpf (and of gametes as well). That is, even if hCCMEDs have partial moral status, their mere availability for research purposes could justify several possibilities regarding the 14-day guideline (Box 1).

Box 1. hCCMED Policy Options for 14-Day Limit

1. The 14-day limit is preserved and applied to hCCMEDs. These entities would be thought to have the same degree of status as human embryos.
2. The 14-day limit is preserved but only applied to human embryos. A lesser moral status could be applied to hCCMEDs and would thus provide an alternative resource for studying human embryogenesis beyond 14 dpf.
3. The 14-day limit is constricted and applied only to research on human embryos. Provided that hCCMEDs at any stage of development are thought to have a lesser moral status than human embryos, they could be used as an alternative to human embryos. They may also be seen as eliminating the need to study human embryos directly for any reason.

The third possibility in Box 1—that hCCMEDs could effectively replace human embryos for all research purposes—is contestable. There might be questions that human embryo research alone could answer, including validating any observations made with hCCMEDs, given the particular dynamics of human embryogenesis from fertilization onward. These experiments would need to be defended scientifically and pass a high degree of ethical scrutiny.

Scientists have argued that experiments with hCCMEDs do not violate the 14-day rule because they are not intact embryos.

Due to the circumstances of their creation, and especially the fact that hCCMEDs do not arise via fertilization, it may be the case that the 14-day rule should not apply to them. This does not eliminate the possibility that considerations about hCCMEDs may influence deliberations about the appropriateness of the 14-day rule, nor does it foreclose the possibility of developing separate guidelines for hCCMED research in light of their morally relevant similarities with actual human embryos.

Policy Issues Raised by hCCMEDs

In relation to the 14-day guideline, hCCMEDs are derived from cultured cells organized to act in embryo-like ways to facilitate the study of early human development. Accordingly, scientists have argued that experiments with hCCMEDs do not violate the 14-day rule because they are not intact embryos. Indeed, part of their promise lies precisely in the fact that researchers can experimentally analyze certain aspects of human embryo development in hCCMEDs in ways that would be impermissible with actual embryos because of the 14-day rule (Aach et al. 2017).

In addition to a lack of federal regulation of human embryo research, the United States also lacks regulation of hCCMEDs. Using the Dickey-Wicker Amendment, the U.S. federal government prohibits funding research utilizing human embryos, but these regulations do not seem to apply to current hCCMEDs because they are considered organized hESCs and not a human organism. In the 2011 case *Sherley vs Sebelius*, the U.S. courts determined that the Dickey-Wicker Amendment prohibited creating and destroying human embryos, but research using existing hESCs could be federally funded (Cuchiara et al. 2013). Therefore, since hCCMEDs involve using existing hESCs and not embryos, they are eligible for federal funding. Moreover, the definition of a fetus in the federal statute requires implantation, which does not occur in current hCCMED models (45 C.F.R. §46.202(c)).

Depending on how embryos are defined, hCCMEDs may also fall outside other national policies. For instance, Italy's policy prohibits the creation of human embryos for research purposes or via cloning, but does not explicitly prohibit hESC research; therefore, hCCMEDs derived from existing hESC lines would be legal (Parlamento Italiano. 2004). Spain defines an embryo as the fertilization of an egg and sperm, and researchers can therefore conduct work on hCCMEDs since they are derived from hESCs (Spain 2007). In

contrast, Austria's ban on human embryos specifies, "cells capable of development may not be used for purposes other than medically assisted procreation," which would include hCCMEDs (Austria National Council 1992).

Currently, the International Society for Stem Cell Research (ISSCR) does not limit hCCMED research to 14 dpf or the primitive streak (ISSCR 2016). It does recommend that research using hCCMEDs, like that using human embryos, should be reviewed by a stem cell research oversight committee (SCRO, sometimes known as Embryo Research Oversight committee, EMRO). By participating in this review process, scientists would be acknowledging the sensitive nature of the research and respecting potential public objections, while still continuing to conduct innovative work. Having a robust ethical review framework also allows for clear classification of the ethical risks associated with the possible developmental trajectories of hCCMEDs both now and in the future. Furthermore, this kind of review provides researchers with confidence that the work they propose has strong scientific and ethical justifications. However, a thoughtful institutional ethical review of hCCMED research might require a higher level of expertise than is generally available in some facilities, which might necessitate outside peer review consultation on the science and ethics of the research.

A thoughtful institutional ethical review of hCCMED research might require a higher level of expertise than is available in some facilities, which might necessitate outside peer review consultation on the science and ethics of the research.

Conclusion

Any reexamination of the 14-day guideline should also consider these embryo-like entities and determine if and when they should fall under human embryo guidelines (ISSCR 2016; Munsie, Hyun, and Sugarman 2017). This assessment is particularly important because it is not clear that current embryo research regulations can be applied to hCCMEDs. As we have seen, hCCMEDs do not go through the usual stages of development (including fertilization). Hence, the 14-day rule may not adequately capture the ways in which these entities might develop morally relevant features (such as a well-differentiated central nervous system) through altered forms of development (Aach et al. 2017; Pera 2015). For instance, the 14-day limit ensures that embryos are not developed beyond the appearance of the primitive streak. But since hCCMEDs might be created from cultured cells at later developmental points (such as a gastruloid that mimics gastrulation, which occurs at 17 dpf), the primitive streak could never happen in culture, with the cells jumping straight to a point beyond this stage.

Furthermore, since hCCMEDs possess a different potential than human embryos, it might be better to develop specialized guidelines for them. Such guidelines could allow for the continuation of valuable research while ensuring that these entities are not manipulated to

develop the potential to turn into an actual human embryo *in utero* or in a permissive *in vitro* environment.

New guidelines could, for example, avoid definitional challenges and instead focus on the characteristics of these entities that generate ethical concerns that are almost identical to those surrounding normal human embryos. For instance, new guidelines could pose limits based on hCCMEDs' functionality, such as the point at which they acquire neural structures that permit the experience of pain, regardless of when they happen (Aach 2017). These suggestions are similar to proposals for alternatives to the 14-day limit of human embryo research. However, there is no consensus even among scientists about what point or feature should be used as the limit on hCCMED research.

With technology and the use of hCCMEDs increasing, it behooves scholars to continue discussions about these entities rather than focus exclusively on human embryo research. At this point, the use of hCCMEDs as an alternative to human embryos allows scientists to acquire knowledge of early human development that they cannot presently obtain by using human embryos. But as research is conducted that can increase the developmental plasticity of hCCMEDs, there should be an effort to explore ethical concerns related to their use as well as discussion about the appropriate level of research oversight.

References

Aach, John, Jeantine Lunshof, Eswar Iyer, and George M. Church. 2017. "Addressing the ethical issues raised by synthetic human entities with embryo-like features." *eLife* 6:e20674. https://elifesciences.org/articles/20674.

Austrian National Council. 1992. "Reproductive Medicine Act (FMedG)." *Federal Law Gazette for the Republic of Austria* (July): 1299-1304. https://www.ris.bka.gv.at/GeltendeFassung.wxe?Abfrage=Bundesnormen&Gesetzesnummer=10003046.

Baylis, Francoise, and Timothy Krahn. 2009. "The trouble with embryos". *Science and Technology Studies* 22 (2): 31-54.

Cuchiara, Maude L., James Lawford Davies, and Kirstin R.W. Matthews. 2013. "Defining 'Research' in the US and EU: Contrast of Sherley v. Sebelius and Brustle v. Greenpeace Rulings." *Stem Cell Reviews and Reports* 9 (6): 743-51.

Deglincerti, Alessia, et al. 2016a. "Self-organization of human embryonic stem cells on micropatterns." *Nature Protocols* 11: 2223-2232.

Deglincerti, Alessi, Gist F. Croft, Lauren N. Pietila, Magdalena Zernicka-Goetz, Eric D. Siggia, and Ali H. Brivanlou. 2016b. "Self-organization of the in vitro Attached Human Embryo." *Nature* 533 (7602): 251-54. https://doi.org/10.1038/nature17948.

Harvard University Embryonic Stem Cell Research Oversight ("ESCRO") Committee. 2018. *Ethical issues related to the creation of synthetic human embryos.*

Hyun, Insoo, Amy Wilkerson, and Josephine Johnston. 2016. "Revisit the 14-day rule." *Nature* 533 (7602): 169-171.

ISSCR (International Society for Stem Cell Research). 2016. "Guidelines for Stem Cell Research and Clinical Translation." Accessed September 21, 2017. http://www.isscr.org/docs/default-source/all-isscr-guidelines/guidelines-2016/isscr-guidelines-for-stem-cell-research-and-clinical-translationd67119731dff6ddbb37cff0000940c19.pdf?sfvrsn=4

Matthews, Kirstin R.W., and Erin H. Yang. 2019. *Politics and Policies Guiding Human Embryo Research in the United States.* Houston: Rice University's Baker Institute for Public Policy. https://www.bakerinstitute.org/media/files/files/a9096889/chb-pub-greenwall-hesc-011519.pdf.

Morgan, Lynn M. 2004. "A Social Biography of Carnegie Embryo No. 836." *The Anatomical Record Part B: New Anatomist* 276B (1): 3-7.

Munsie, Megan, Insoo Hyun, and Jeremy Sugarman. 2017. "Ethical issues in human organoid and gastruloid research." *Development* 144(6): 942-5.

Parlamento Italiano. 2004. "Rules on medically assisted procreation." *Official Gazette,* no. 45 (February): 1-5. http://www.camera.it/parlam/leggi/040401.htm.

Pera, Martin F., et al. 2015. "What If Stem Cells Turned into Embryos in a Dish?" *Nature Methods* 12 (10): 917-19.

Protection of Human Subjects. 2001. 45 C.F.R. §46.202(c). "Subpart B. Additional Protections for Pregnant Women, Human Fetuses and Neonates Involved in Research." Accessed January 30, 2019. https://www.hhs.gov/ohrp/regulations-and-policy/regulations/45-cfr-46/index.html#46.202.

Rivron, Nicolas C., et al. 2018. "Blastocyst-like structures generated solely from stem cells." *Nature* 557: 106.

Shahbazi, Marta N., et al. 2016. "Self-organization of the Human Embryo in the Absence of Maternal Tissues." *Nature Cell Biology* 18 (6): 700-08. https://doi.org/10.1038/ncb3347.

Simunovic, Mijo, and Ali H. Brivanlou. 2017. "Embryoids, organoids and gastruloids: new approaches to understanding embryo genesis." *Development* 144 (6): 976-85.

Spain. 2007. "Law on Biomedical Investigation." https://www.boe.es/boe/dias/2007/07/04/pdfs/A28826-28848.pdf.

Van den Brink, Susanne C., et al. 2014. "Symmetry Breaking, Germ Layer Specification and Axial Organisation in Aggregates of Mouse Embryonic Stem Cells." *Development* 141: 4231-42.

Warmflash, Aryeh, Benoit Sorre, Fred Etoc, Eric D. Siggia, and Ali H. Brivanlou. 2014. "A Method to Recapitulate Early Embryonic Spatial Patterning in Human Embryonic Stem Cells." *Nature Methods* 11 (8): 847-854.

Warren, Mary Anne. 1997. *Moral Status: Obligations to Persons and Other Living Things.* Oxford: Oxford University Press.

Wu, Haojia, Kohei Uchimura, Erinn L. Donnelly, Yuhei Kirita, Samantha A. Morris, and Benjamin D. Humphreys. 2018. "Comparative Analysis and Refinement of Human PSC-Derived Kidney Organoid Differentiation with Single-Cell Transcriptomics." *Cell Stem Cell* 23 (6): 869-881. https://doi.org/10.1016/j.stem.2018.10.010.