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Toward Guidelines for Research on Human Embryo Models Formed from Stem Cells

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Over the past few years, a number of research groups have reported striking progress on the generation of *in vitro* models from mouse and human stem cells that replicate aspects of early embryonic development. Not only do these models reproduce some key cell fate decisions but, especially in the mouse system, they also mimic the spatiotemporal arrangements of embryonic and extraembryonic tissues that are required for developmental patterning and implantation in the uterus. If such models could be developed for the early human embryo, they would have great potential benefits for understanding early human development, for biomedical science, and for reducing the use of animals and human embryos in research. However, guidelines for the ethical conduct of this line of work are at present not well defined. In this Forum article, we discuss some key aspects of this emerging area of research and provide some recommendations for its ethical oversight.

The Current State of Research into Embryo Models from Stem Cells

Beginning with fertilization, the mammalian conceptus (see Box 1 for terminology) goes through a characteristic developmental program with two essential outcomes: (1) the formation of extraembryonic tissues that establish connections with the maternal tissue and fuel the development of the embryo proper and (2) the generation of the three embryonic germ layers of cells that are the building blocks of the future organs. These two processes lay the groundwork for the developing embryo to form a fetus.

Although the mammalian conceptus can develop *in vitro* up to the blastocyst stage, *in vivo* studies are particularly challenging once implantation in the uterus occurs (approximately day 7–9 in humans), due to the small size of the conceptus and its inaccessibility in the uterine tissues. Protocols allowing for the further *in vitro* development of mouse blastocysts were developed starting 48 years ago (Hsu, 1971, 1979), but only recently were these applied to donated fertility clinic-derived human blastocysts (Deglincerti et al., 2016). These *in vitro*-cultured human blastocysts reflect some aspects of early post-implantation stages (Bo et al., 2019; Deglincerti et al., 2016; Shahbazi et al., 2016), but their growth is largely confined to two dimensions, they do not yet fully mimic *in vivo* development, and they often show degenerative changes as culture progresses. Three-dimensional cultures of human blastocysts are now being developed that better recapitulate post-implantation development *in vitro* (Xiang et al., 2019). However, the optimization of culture conditions and the extension of the development of such cultures risk contravening the internationally well-accepted 14-day rule, which limits research on intact human conceptuses/embryos (see below for definition of human embryo in law) to 14 consecutive days of development *in vitro* from fertilization or the appearance of the primitive streak, whichever occurs first. Given these limitations, the possibility of using human stem cells to generate *in vitro* models of early development represents an alternative. These models have important technical advantages, including accessibility (cell lines are more widely available than blastocysts and represent a renewable resource) and the ability to generate large numbers required for high-throughput screens and complex gene editing. A range of different and complementary models, based on several types of methodology, has been devised to mimic specific stages of mouse and human development (Figure 1) (Beccari et al., 2018; Rivron et al., 2018b; Shao et al., 2017; Sozen et al., 2018; Warmflash et al., 2014).

Although none of these models has yet been demonstrated to be competent to develop for more than a few days *in vitro*, we can envision that cell culture methodologies could be refined to a point where the models capture key features of early mammalian development with sufficient fidelity to minimize differences from the conceptus itself. When applied to human cells, such refinements will greatly enhance the power of the models but will also elevate ethical concerns over the conduct of the research.

Box 1. Definition of Terms

- Conceptus: the products of conception at all stages of development from zygote to birth. These include the embryo proper, the fetus, the placenta, and all extraembryonic membranes. The term "embryo proper" refers to those parts of the conceptus that will form the new body and excludes the extraembryonic tissues. Often, the terms "embryo" and "conceptus" are used interchangeably.
- Pre-implantation stages of development: the first few days of development, from fertilization to implantation, during which the conceptus travels down the oviduct toward the uterus. It encompasses the 7–9 days after fertilization in humans.
- Implantation: the process of attachment and invasion of the conceptus to the uterine tissues that occurs around day 7–9 after fertilization in humans. Implantation establishes the fetal-maternal interface leading to later placental development. Implantation is mediated by the polar (embryonic side near the epiblast) and mural trophectoderm cells (abembryonic side opposite the epiblast) of the blastocyst.
- Post-implantation stages of development: the stages of development after the conceptus is embedded in the uterine tissues.
- Gastrulation: the process by which the three germ layers of the embryonic compartment of the conceptus are formed. Gastrulation begins around day 14 in humans.
- Primitive streak: the embryonic structure that establishes bilateral symmetry (alignment of equivalent structures on both sides of the anterior-posterior axis), the site of gastrulation, and the formation of the germ layers. In humans, the primitive streak appears after 14 days.
- Embryonic and fetal stages: the embryonic stage begins with the division of the zygote and encompasses the development of the body plan and formation of the organs. This is followed by the fetal stage, during which growth and maturation of tissues and organs occurs. In humans, the fetal stage begins during the 9th week after fertilization and continues to birth.

Potential Benefits of This Research to Human Health

This research could result in substantial scientific and medical advances with an impact on human health. These outcomes include

- attaining a fundamental understanding of key stages of early human development through the availability of versatile and scalable experimental models;
- achieving a better understanding of how stem cells from different species (e.g., mouse, pig, human) and different cell lines and states vary in developmental potential, to improve their use in research and therapy;
- improving assisted reproduction technologies;
- understanding the biology of germ cells and infertility;
- modeling implantation in three dimensions to understand the high early pregnancy loss and improve contraception technologies;
- studying the genetics of early developmental defects using gene editing technologies;
- revealing how defects in epigenetic reprogramming during early development can influence health throughout the lifespan, leading to strategies for disease prevention according to the developmental origins of health and disease;
- refining human stem cell differentiation culture methods to achieve a greater fidelity with processes occurring during development (e.g., organogenesis).

The Current State of Regulation of Research on Human Embryo Models from Stem Cells

Since this field is relatively new, there is little explicit regulation of *in vitro* human embryo models. However, research using certain models of early human development may fall under existing legislation depending on how a human embryo is defined. Legal definitions of a human embryo vary greatly across jurisdictions and are not necessarily restricted to entities with the potential to form a full organism (Box 2). In other jurisdictions, legislation pertaining to human embryos may not include an explicit definition, leading to uncertainty around how models of human development would be viewed.

Moreover, this consideration of developmental or "organismic" potential (see the International Society for Stem Cell Research [ISSCR] guidelines, Box 3) is problematic in the human, because the definitive experiments to prove or disprove full human developmental potential are obviously not possible under accepted ethical standards. Also, previous experience (e.g., somatic cell nuclear transfer), along with knowledge about species differences, showed that findings in one specific species do not automatically translate to the human case. Thus, the developmental competence of a

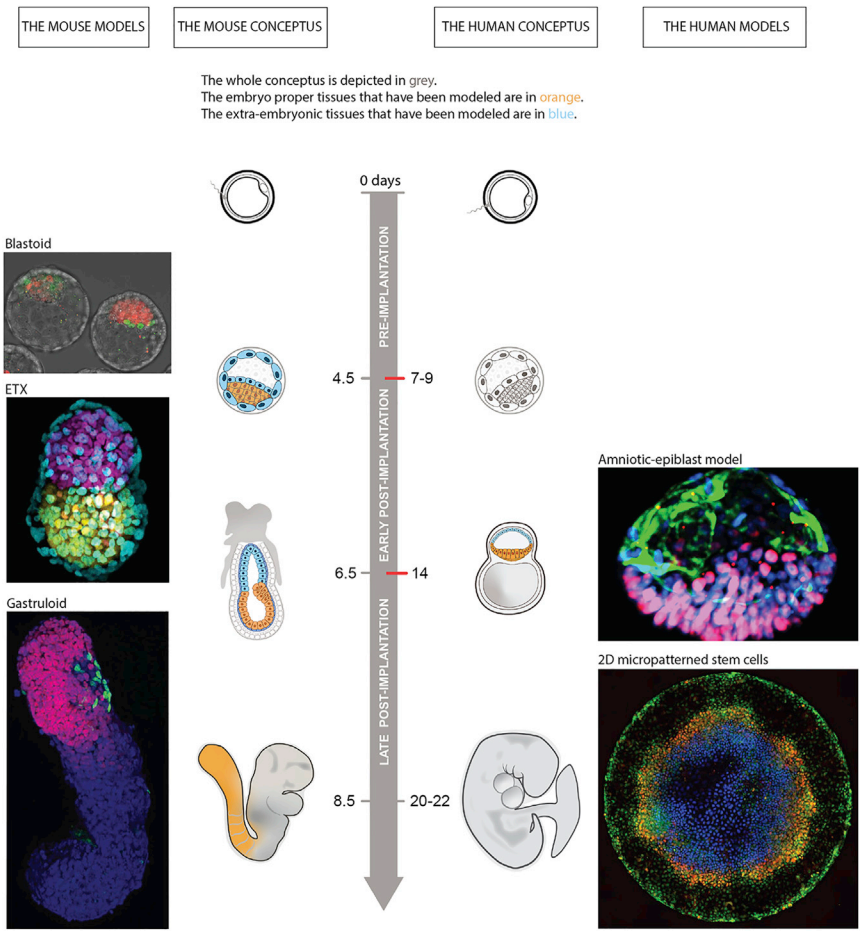

Figure 1. The Different Embryo Models

Models of the embryo have been formed using mouse (left) and human stem cells (right) to mimic the development of (parts of) the embryo proper (orange) and the extraembryonic tissues (blue) of the conceptus (gray). Mouse models include blastoids (left, top) that resemble the pre-implantation 3.5-day-old conceptus, contain analogues of the three lineages forming the embryo proper, placenta, and yolk sac, and recapitulate aspects of the implantation into the uterus; ETX embryo models (left, middle) that resemble inner regions of the early post-implantation 6.5-day-old conceptus, contain analogues of the three lineages forming the embryo, placenta, and visceral endoderm, mimic an anteroposterior patterning, and form gastrulating-like cells; and gastruloids (left, bottom) that resemble the medial and posterior parts of the 8.5-day-old embryo proper form features of the three orthogonal axes that serve as a reference for the organization of the derivatives of the three germ layers and an appropriate distribution of the primordia-like cells. Work with human stem cells is less advanced but is on a similar trajectory. Currently, epiblast-amniotic models (right, top) recapitulate features of the formation of the amniotic cavity, epiblast-amniotic ectoderm axis, and gastrulation, while human stem cells grown on 2D micropatterns (right, bottom) model aspects of post-implantation patterning. Days 7–9 and day 14 of human development (marked in red) are important biological milestones that delineate the emergence of specific properties, such as the capacity to implant in the uterus and the formation of the primitive streak (gastrulation), respectively.

model of one species (e.g., the mouse) is not automatically informative regarding what could occur in the human species. In addition to uncertainty regarding how to define organismic potential in embryo models, it is also difficult to know how to apply the 14-day rule to such systems. Stem cells derived from human blastocysts or obtained via reprograming do not have a defined developmental chronology per se (days since fertilization) because cultures are not precisely constrained *in vitro* and comprise a spectrum of concomitant developmental states. For example, human pluripotent stem cells grown in 3D microfluidic devices can form structures resembling the early primitive streak stage within 48 h of culture. Also, defining the appearance of specific anatomical structures as a landmark, such as the appearance of a primitive streak, can be prone to interpretation and can be ascertained only after the culture is terminated. Finally, both embryos and *in vitro* models show variability in developmental chronology and may be more advanced or delayed relative to the average timeline. Altogether, this uncertainty in the developmental timing creates difficulties for the application of ethical limits, such as the 14-day rule, to embryo models.

Currently, there is little in the way of specific guidelines to provide for proper ethical oversight of this research, despite the substantial scientific and medical insights to be gained (Rivron et al., 2018a). It is thus critical that any discussions on the future of this research be carried out openly and on a scientifically informed basis. To this end, we wish to clarify some ethical issues relative to different embryo models and put forward some suggestions regarding guidelines for the conduct of this research.

General Ethical Considerations Regarding Embryo Models

1. Constructs that do not attempt to model the integrated development of the entire conceptus are not equivalent to embryos. This includes research that examines single embryonic or extraembryonic cell lineages (e.g., epiblast, trophoblast, or

Box 2. Examples of Jurisdictions in which the Definition of a Human Embryo Might Capture Embryo Models Derived from Stem Cells

Country	Legal Definition of a Human Embryo	Citation
Australia	"A discrete entity that has arisen from either: (a) the first mitotic division when fertilisation of a human oocyte by a human sperm is complete; or (b) any other process that initiates organised development of a biological entity with a human nuclear genome or altered human nuclear genome that has the potential to develop up to, or beyond, the stage at which the primitive streak appears; and has not yet reached 8 weeks of development since the first mitotic division."	Prohibition of Human Cloning for Reproduction and the Regulation of Human Embryo Research Amendment Act 2006, no. 172, 2006, http://www.comlaw.gov.au/Details/C2006A00172.
Japan	Article 2 (1) "(i) Embryo—A cell (except for a Germ Cell) or a cell group which has the potential to grow into an individual through the process of development in utero of a human or an animal and remains at a stage prior to placental formation."	Act on Regulation of Human Cloning Techniques (Act no. 146 of 2000), http://www.cas.go.jp/jp/seisaku/hourei/data/htc.pdf.
United States	SEC. 509. "(a) None of the funds made available in this Act may be used for— (1) the creation of a human embryo or embryos for research purposes; or (2) research in which a human embryo or embryos are destroyed, discarded, or knowingly subjected to risk of injury or death greater than that allowed for research on fetuses in utero under 45 CFR 46.204(b) and section 498(b) of the Public Health Service Act (42 U.S.C. 289g(b)). (b) For purposes of this section, the term 'human embryo or embryos' includes any organism, not protected as a human subject under 45 CFR 46 as of the date of the enactment of this Act, that is derived by fertilization, parthenogenesis, cloning, or any other means from one or more human gametes or human diploid cells."	Dickey-Wicker Amendment, 2009, https://www.congress.gov/bill/111th-congress/house-bill/1105/text/enr. [This amendment prohibits the NIH from funding human embryo research as defined here.]

extraembryonic endoderm) or combinations of embryonic and extraembryonic lineages that are not likely to support the integrated development of an intact embryo (e.g., gastruloid, epiblast-amniotic sac structures). In these cases, we can safely infer from experimental studies in mice that such constructs are not able to develop independently into embryos or fetuses absent further complementation with all extra-embryonic tissues. Thus, gastruloids, epiblast-amniotic sac constructs, and micropatterned cultures can provide useful models without equivalent ethical concerns.

2. Experimental constructs that attempt to model the integrated development of the entire conceptus need to be carefully assessed. Without extraembryonic tissues, the *in vivo* development of the human conceptus cannot proceed normally. Because the trophectoderm of the blastocyst enables the process of *in utero* implantation, embryo models that combine cells mimicking the epiblast and extraembryonic endoderm and covered by trophectoderm-like cells (e.g., blastoids) do raise the possibility of producing a construct with further potential if transferred to the human uterus. Current research in mice suggests that the extended developmental potential of such models is far from certain at this point.
3. Regulation of research on human embryo models that do attempt to model the integrated development of the entire conceptus should be informed by the current approach to regulation of embryo research. Depending on the jurisdiction, research is currently permitted, under appropriate oversight, on

Box 3. Sections of Current International Society for Stem Cell Research Guidelines Relevant to Embryo Models

FORMS OF RESEARCH THAT ARE PERMISSIBLE ONLY AFTER REVIEW BY AN EMRO PROCESS

Research involving the *in vitro* culture of embryos or experimental generation of embryo-like structures that might manifest human organismal potential, to ensure minimal periods of *in vitro* culture, as justified by compelling scientific rationale.

PROHIBITED RESEARCH ACTIVITIES

a. *In vitro* culture of any intact human pre-implantation embryo or 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.
b. Experiments whereby human embryos or organized cellular structures that might manifest human organismal potential are gestated *ex utero* or in any non-human animal uterus.

established lines of human stem cells, on parts of embryos, and on pre-implantation embryos developed up to 14 days. Appropriate mechanisms for review of such research exist in most jurisdictions, e.g., Embryo Research Oversight (EMRO) in the United States, Human Fertilization and Embryo Authority in the United Kingdom, Stem Cell Oversight Committee in Canada, and Embryo Research Licensing Committee in Australia.

4. Support of this research through peer-reviewed funding by public agencies and charitable foundations is critical to ensure proper scientific and ethical oversight.
5. Regulation should take into account the intent of the research. Human embryo models should not be created for direct use in assisted reproduction aimed at producing a pregnancy but could be used as *in vitro* models to develop improvements to the management of human reproduction.
6. Oversight should consider both the potential biomedical benefits of the work and its scientific quality. Oversight and approval should take into account the ethical and practical benefits of replacing human embryo material with broadly available stem cell lines. However, the scientific rationale and quality of the proposed research need to be carefully evaluated.

Recommendations for Oversight of Research on Specific Model Systems

1. Culture systems that model pre-implantation development and post-implantation development up to gastrulation by incorporating human embryonic and extraembryonic lineages, including the trophoblast and extraembryonic endoderm, with the intent to represent the integrated development of the entire conceptus up to the appearance of the primitive streak (for example, blastoids or ETX models) are permissible only following oversight by EMRO in the United States or equivalent ethics review elsewhere (or more extensive review where local regulations require it).
2. 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 but are notifiable to the EMRO or equivalent and subject to review should the cognizant body deem it necessary.
3. Culture systems that model human gastrulation and subsequent stages (beyond the appearance of the primitive streak) are exempt from mandatory review if they do not encompass all major lineages of the conceptus (embryonic and extraembryonic) in an intact construct and are aimed at studying a discrete and defined period of development or discrete set of anatomic structures, rather than modeling the continuous development of an intact embryo or fetus (for example, models of the neural tube or micropatterned stem cell cultures forming three germ layers, or gastruloids). Such research would be notifiable to the EMRO or equivalent and subject to review should

the cognizant body deem it necessary.

4. A human embryo model that was disassembled at the time of appearance of the primitive streak into component parts for further culture or study *in vitro* would no longer be subject to the strict considerations suggested in (1) and may be determined to be exempt from further committee review.
5. The *in vitro* combination of human embryo models with animal or human cells or tissues or embryos should be subject to the same limitations in (1) and (2) above and mandatory review. This category would include *in vitro* human/animal embryo model chimeras, embryo model/human embryo chimeras, or any of these constructs implanted *in vitro* into explanted uterine tissues or uterine organoids.
6. No human embryo model in any of the above categories shall be transferred *in vivo* into the uterus of an animal or human.

Next Steps

Research in this area is evolving rapidly and the support of this research by public agencies and charitable foundations is critical to ensure proper scientific and ethical oversight. An open, broadly based consultative process needs to occur so that a regulatory framework can be carefully developed. Any proposed regulations should be clear and take into account the potential benefits of this research and the unique power of these models. Publicly accountable regulatory oversight is preferable to proscriptive legislation that could have unintended negative consequences in the future. The ISSCR is in a strong position to make authoritative recommendations for the conduct of this research, as it has done in the past for other ethically challenging issues within its remit. The current ISSCR guidelines (ISSCR 2016 Guidelines for Stem Cell Research and Clinical Translation http://www.isscr.org/membership/policy/2016-guidelines/guidelines-for-stem-cell-research-and-clinical-translation, Box 3) provide some baseline recommendations relevant to this research but need to be clarified and expanded in the light of the rapid progress in this field. We put forward our recommendations as a starting point for discussions within the Society and the community at large.

AUTHOR CONTRIBUTIONS

All the authors contributed equally to this work and are listed in alphabetic order.

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