

## SPOTLIGHT

# Human embryo research and the 14-day rule

Martin F. Pera\*

## ABSTRACT

In many jurisdictions, restrictions prohibit the culture of human embryos beyond 14 days of development. However, recent reports describing the successful maintenance of embryos *in vitro* to this stage have prompted many in the field to question whether the rule is still appropriate. This Spotlight article looks at the original rationale behind the 14-day rule and its relevance today in light of advances in human embryo culture and in the derivation of embryonic-like structures from human pluripotent stem cells.

## Introduction

In a number of jurisdictions, *in vitro* culture of the human embryo is not allowed to proceed beyond the equivalent of day 14 of embryonic development, or the approximate time at which the primitive streak appears. Recent studies have described the successful growth of human embryos to a chronological and developmental stage approaching this limit (Deglincerti et al., 2016; Shahbazi et al., 2016), and other work has reported the generation of embryo-like structures from mouse and human pluripotent stem cells that mimic the gastrulating embryo in form and cellular content (Etoc et al., 2016; Harrison et al., 2017; Shao et al., 2017; van den Brink et al., 2014). These advances have prompted a re-examination of the current restrictions on human embryo culture (Hyun et al., 2016). Making changes to standing legislation is a complex and arduous process, however, and one that risks engendering new strictures that could have unintended consequences for future research. Is an immediate campaign to alter the 14-day rule either necessary or desirable for the advancement of our knowledge of human embryology?

## The origins of the 14-day rule

The 14-day rule had its origins in bioethical discussions that took place in the early days of the *in vitro* fertilisation (IVF) field in the 1970s. The first IVF pregnancy was reported in 1973 by the team of Carl Wood at Monash University in Australia (De Kretzer et al., 1973), and the first successful birth of a child conceived by IVF was achieved by Steptoe and Edwards in the UK in 1978 (Steptoe and Edwards, 1978). Throughout this period, bioethicists and theologians contemplated the vexing question of the moral status of the human embryo. The 14-day rule was never meant to answer that question, but its formulation was undoubtedly influenced by the discourse surrounding it.

The first public document to recommend a 14-day limit to the growth of the human embryo *in vitro* was the report of the Ethics Advisory Board of the (then) Department of Health Education and Welfare in the United States (HEW Support of Research Involving Human In Vitro Fertilisation and Embryo Transfer, 1979). In 1984,

the UK Committee of Inquiry into Human Fertilisation and Embryology (the Warnock Committee) endorsed the 14-day limit, and expounded further on the rationale for its implementation (Report of the Committee of Inquiry Into Human Fertilisation and Embryology). Four key arguments were noted in support of a 14-day limit on embryo culture: (1) 14 days is the last stage in development at which twinning can occur and therefore represents the point of individuation, (2) not even the founding cells of the nervous system have been specified prior to this stage, (3) there is substantial embryo loss from the time of fertilisation up to this point, and (4) until the process of implantation is complete, the embryo has no potential for further development. Ongoing reconsideration of the scientific basis for some of these arguments would certainly be valuable; for instance, concepts concerning the mechanisms of twinning and the timing of origin of twin embryos remain inferential owing to a paucity of model systems available for study (Hall, 2003).

Interestingly, the Warnock Committee, who were unusually prescient in their consideration of future scientific developments, discussed the possibility of ectogenesis, or ‘developing embryos in an artificial environment for progressively longer periods’, making it possible ‘to study in detail normal and abnormal human development at the embryonic and foetal stages’. The Committee conceded that such a technique could arouse ‘much anxiety’, but held that such scientific developments ‘were beyond the time horizon within which this inquiry feels it can predict’ and that in any case, their firm recommendation was that ‘growing of a human embryo *in vitro* beyond 14 days should be a criminal offence’. Arguably, we have arrived on the edge of the horizon that the Warnock committee foresaw, and current research is running headlong into prohibitions established at a time when embryonic stem cells and even the culture of the human embryo to the blastocyst stage both lay in the long-term future.

## The potential benefits and feasibility of longer-term embryo culture

Any argument for the propagation of embryos or embryo-like structures beyond the current 14-day limit should make very clear the potential medical benefits of the research. Unlike mitochondrial replacement therapy, another recent embryological advance that gave rise to widespread debate and ultimately to changes to legislation, post-implantation embryo research is not likely to provide a direct route to a treatment for disease. Its potential medical impact is, however, very substantial. Contributions from such research could include advancement of our knowledge of gene function during embryogenesis; a scientific foundation for prevention of early pregnancy loss, birth defects and teratogenesis; an understanding of how the widespread epigenetic programming that occurs during this stage of development might impact disease progression in later life; and refinements to the fidelity with which pluripotent stem cell differentiation mimics embryogenesis, with anticipated improvements in the efficient production of differentiated cells with the desired functional capacity for research and therapy.

The Jackson Laboratory, 600 Main Street, Bar Harbor, ME 04609, USA.

\*Author for correspondence (martin.pera@jax.org)

 M.F.P., 0000-0001-6239-0428

Although the potential medical benefits of such research are significant, it is less certain whether extending the 14-day limit on human embryo culture would be the best route towards realising them. The possibility for extended normal development of human embryos *in vitro* through and beyond the primitive streak stage is unknown. In the mouse, pre-implantation embryo culture has not, at least to date, generated structures resembling post-implantation embryos, in contrast to the recent results with stem cell cultures. In studies thus far, human embryo cultures have shown degenerative changes as they approach the 14-day limit using current methodology. Mouse post-implantation embryos can be grown *in vitro* to a stage equivalent to embryonic day 10.5 (about 4 weeks of human development), but it is obviously unethical to harvest post-implantation embryos from the human. The question then becomes whether it is feasible to instead extend the development of high quality human embryos *in vitro* to this stage. Even if it were, however, the extremely limited supply of such embryos means that any line of research that requires large numbers of cells would be extremely difficult to perform and reproduce across multiple laboratories.

Additional questions would arise over the extent to which human post-implantation embryo development culture replicates embryogenesis *in vivo*. For human pre-implantation stages, we know that human IVF culture can routinely support growth of embryos capable of development to term, a feature which in and of itself provides substantial validation of the experimental system. There will be no such guarantees for the post-implantation embryo culture, so alternative approaches, such as transcriptome or epigenome analysis, will be required to assess whether cultured cells do indeed show a similar phenotype to cells in embryos. Because reference to early human post-implantation development is not possible, we will rely instead on comparisons with emerging studies in non-human primates.

### Human embryo-like structures: an alternative to bona fide human embryo research

In almost all jurisdictions, research on the human embryo is only permitted if the goals of the research cannot be achieved through other means. Recent advances in the generation of embryo-like constructs derived from human pluripotent stem cells could provide an alternative to the use of embryos, and a possible way around using human embryos generated through IVF. It is already clear that three-dimensional cultures of cells derived from human pluripotent stem cells are capable of extensive organotypic morphogenesis, forming structures that resemble the developing eye, cortex, or kidney and other tissues (Clevers, 2016). These studies are at an early stage, comparable to the beginnings of embryonic stem cell research, when researchers relied on spontaneous and uncontrolled cell differentiation in adherent cultures or embryoid bodies to generate specialised cells. Since then, directed differentiation protocols, which are largely based on embryological principles and emulate normal developmental pathways, have facilitated the large-scale production of a range of cell types for research and therapy, mostly in two-dimensional culture platforms. It is not difficult to envision that this directed differentiation could be implemented in three-dimensional cultures by engineering microwells, microfluidic chambers, and smart surfaces to yield spatially and temporally controlled delivery of growth factors and extracellular matrices, thus reconstructing the embryonic environment to promote key morphogenetic processes (Murrow et al., 2017). Today, single-cell transcriptional profiling enables comparison of stem cell cultures *in vitro* to the human pre-implantation embryo, non-human primate post-implantation

stages, and human embryonic and foetal tissues at later stages of development (4–5 weeks onward), allowing validation of the culture model. Improvements in our ability to expand stem cell culture have paved the way to large-scale, highly parallel experimentation. CRISPR-Cas9 editing now allows for facile introduction of specific genetic and epigenetic modifications onto a constant genetic background. In short, technical advances over the past several years have revolutionised our capacity to exploit stem cells to study development.

The generation of human embryo-like structures from stem cells *in vitro* could, of course, itself raise significant ethical issues (Aach et al., 2017; Pera et al., 2015). In many jurisdictions, it is not clear whether these laboratory constructs would be captured under current legislation, owing to the manner in which embryos are defined in law. Indeed, for future *in vitro* research, the definition of an embryo, rather than the time limit on its propagation, might be the most important regulatory question. If we think of a mammalian embryo as an entity capable of continuous and integrated development towards live birth, then experimentally produced constructs that aim to duplicate only a particular stage of development or a particular anatomical structure would likely be excluded from such a definition. Much informative work would fall into this category. In the end, the extent to which embryo-like structures made from stem cells actually resemble embryos will determine the nature of the bioethical issues that arise from their creation and use. Such questions might be best addressed on a project-by-project basis, with deliberations guided by fundamental principles established by national or international bodies following widespread discussion and public consultation. Precedent and infrastructure for this approach exists in the form of Stem Cell Research Oversight Committees, who oversee scientific and ethical aspects of conformance with national and international standards. Such a regulatory arrangement, implemented with public accountability, is surely preferable to proscriptive legislation, which cannot predict or respond to future scientific developments in a rapidly moving field.

### Concluding remarks

It might turn out that the only way to study human post-implantation development is to study post-implantation embryos, and that would indeed require changes to the current legislation that locks in the 14-day rule in a number of jurisdictions. In the meantime, there is much groundwork that can be done by refining three-dimensional culture technology using pluripotent stem cells, and through the careful study of limited numbers of non-human primate embryos. It seems likely that supply and access will inevitably place constraints on what can be achieved with human embryos. Part of the rationale originally put forward to support the derivation of human embryonic stem cells was their potential as models for human development. The prospects for realising this potential have never been brighter.

### Competing interests

The author declares no competing or financial interests.

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