“The 14-day rule”

SCAAC

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Robin Lovell-Badge

The Francis Crick Institute
1 Midland Road, London NW1 1AT, UK

robin.lovell-badge@crick.ac.uk

The 14-day rule: history

- The 14-day rule has both governed and enabled research on early human embryos for about 40 years
- It was suggested in the USA, but developed by the Warnock Committee in 1984, notably by Anne McLaren.
- The rule, which is the limit on how long intact human embryos could be cultured *in vitro*, is actually:

  "14 days or the first appearance of the primitive streak."

- It was proposed as a compromise to permit some embryo research in the face of strong opposition, particularly from religious groups.
- Scientifically: 14 days is prior to any specification of the early central nervous system, indeed weeks before the first neurons appear, and months before any connectivity and sensory systems are present. It is therefore a long time before consciousness or an ability to feel pain develops.
- Practically: It *was* a safe limit at the time with no methods available to go beyond 7 days.
- Religiously: Given that monozygotic (identical) twins can form from splitting of an embryo at any time up until gastrulation, and that individuals can't have the same soul, it was suggested that 'ensoulment' must occur after 14 days.
- Legally: Having a rule based on a number like 14 days is very simple for everyone to understand and to operate.

The 14-day rule – legislation and guidelines

- In the UK, the 14-day rule was brought into guidelines in the UK and then into law, under the Human Fertilisation and Embryology Act (HFEA) in 1990.

- It was rapidly adopted either in law or in guidelines by many countries, and it has been in international guidelines, notably those of the ISSCR since these were first proposed.

- Of the 22 top science and technology R&D intensive countries, 12 have a 14-day limit; one, Switzerland, has a 7-day limit and five prohibit human embryo research.

- Four countries have no specific 14-day limit or other legal restriction on human embryo research, including the USA (although some states have relevant laws).

[Matthews and Morali (2020) Regenerative Medicine 15]

Why keep the 14-day rule ?

- It has been argued that it has been a valuable ‘contract’ between researchers and those opposed to human embryo research with a clear understanding of boundaries that have operated well for many years. Breaking this ‘contract’ may upset the trust the people have in scientists.

- Why ‘rock the boat’ and risk opponents of research (whose views have not evolved), influencing politicians and restricting our ability to do anything ?

- We still don’t know enough about what happens between 7 and 13 days – why not focus studies on this period ?

- It is possible to use alternatives to human embryos to study post-14 day development, such as animal embryos or stem cell-based embryo models.

Why change the 14-day rule ?

- Many studies suggest that important events taking place during early embryogenesis, even prior to 14 days, are likely to have an impact on later development.
- But without the ability to go further, their real significance will be hard to ascertain. Moreover, the embryo itself, as opposed to some of the supporting extraembryonic tissues, only starts to develop after 14 days.
- How are we to learn about our beginnings if we cannot study them?
- We now have methods that will allow culture to at least 13 days – probably longer based on the work with NHPs – we can benefit from these.
- Greater understanding of normal human development during and just following gastrulation, about which we know little, and of congenital defects that may arise during this period (e.g. heart and CNS defects), as well as to reduce rates of miscarriage.
- It would also allow better testing of the safety (and efficiency) of new techniques, such as those allowing mitochondrial replacement (MRT), the use of in vitro derived gametes, and heritable genome editing. Even testing culture methods used in IVF may benefit.
- With stem cell-derived embryo models, “14 days” becomes a meaningless limit.

Why change the 14-day rule ?

- The period between 7 and 28 days or perhaps now between 14 and 28 days is referred to as the black box period.

- All we know about normal human embryo development comes from the Carnegie series of human embryos (fixed tissue) and from embryos studied after terminations – which are almost always after 28 days.

- Shankar Srinivas was able to obtain one embryo from a very early termination, which was estimated by comparisons with the Carnegie series to be about 16-18 days.
  - There was no neuroectoderm – what does this say about the reasons behind adopting 14 days as the limit ?
  - While this embryo gave valuable information, it was only one.

Why change the 14-day rule ?

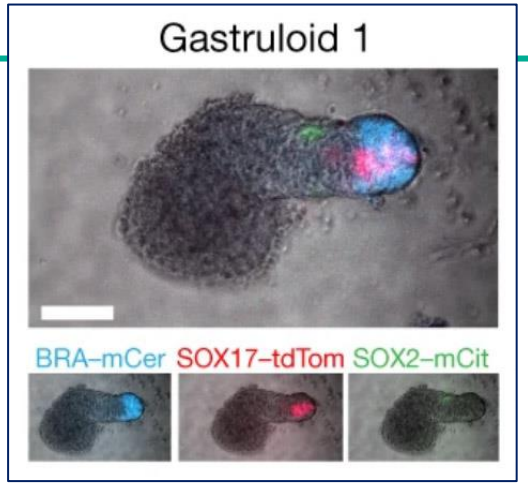
What about animal embryos ?

- It is now clear that there are many differences between gene activity, the role of specific pathways, cell types and processes between most animal models and human embryos.
- NHP embryos may be closer to human embryos, but:
  - (a) we will never know without doing the comparison.
  - (b) there are ethical issues of using NHPs in research.

What about stem cell-based embryo models ?

- It appears that these may be good at modelling some aspects of normal human development and they could be very useful.
- However, without validation involving direct comparisons with intact human embryos in culture (or obtained from early terminations) how will we ever know ?
- If this validation can be done, then it would be possible to reduce the numbers of human embryos used in research (although not eliminate this).

Early Embryo Models derived from ES or iPS cells



Moris, et al, Martinez Arias *Nature*, June 2020

Non-integrated stem cell based embryo models

- Models that experimentally recapitulate some, but not all aspects of the post-implantation embryo, such as “Gastruloids”.
- These have been very useful for studying aspects of development, such as somitogenesis and posterior spinal cord formation.

N.B. These don’t undergo gastrulation and they tend to lack anterior structures, notably the brain. However, they can have beating hearts, etc.

Integrated stem cell-based embryo models

- Models that contain a range of embryonic and extra-embryonic structures and could potentially achieve the complexity to undergo events of early organogenesis and placental formation *in vitro*. Blastoids are an example of these.

It is not known if these could implant in a uterus and undergo normal development – so far it is thought they are not quite good enough. (This would have to be demonstrated in an animal.)

However, their usefulness as a model relies on them being as close as possible to normal embryos.

Stem cell-derived embryo model policies

- Thirteen countries have definitions of embryo in a national law or guideline:

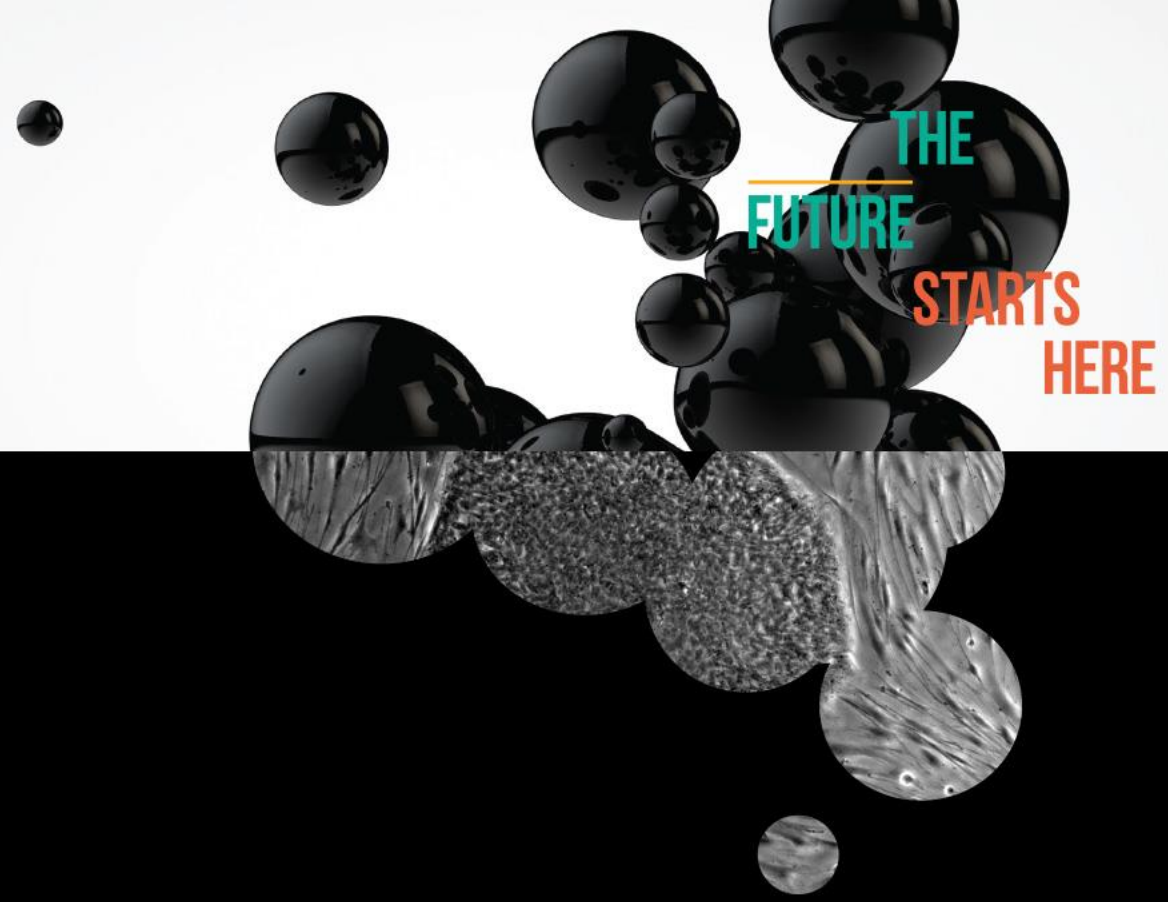
  Australia, Belgium, Canada, Germany, India, Japan, the Netherlands, South Korea, Spain, Switzerland, Taiwan, UK and the USA.

- Human embryo definitions vary, as does their potential impact on embryo model research, with some (Canada) permitting research, while others (Australia) prohibit it.

  [*Matthews and Morali (2020) Regenerative Medicine 15]*

- The UK has not considered stem cell-derived embryo models as falling under the HFE Act. They regulate research on derivation of ES cell lines, but not their use (MRC Stem Cell Bank), *and they do not regulate work with iPS cells*.

ISSCR 2021 VIRTUAL



THE ISSCR GUIDELINES

2021 UPDATES

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ISSCR Guidelines

Scientific and ethical rigor, independent oversight, and transparency are the core values that the Guidelines apply to all aspects of stem cell research and translation. The Guidelines:

- Outline an imperative principle that scientifically meritorious but novel stem cell research projects undergo a specialized oversight process.
- Provide confidence to researchers, clinicians, and the public alike that stem cell science can proceed responsibly and remain responsive to public and patient interests.
- Serve as a basis for regulation and oversight of research worldwide and guide the implementation of new regulatory frameworks in countries establishing support for this advancing research.

Specialized Oversight Process

- The specialized scientific and ethics oversight process must include an assessment of the:
  - scientific rationale and merit of research proposals
  - relevant expertise of the researchers
  - lack of a justifiable alternative
  - ethical permissibility
  - legal permissibility within the jurisdiction
  - justification for the research
- The process should be conducted by qualified scientists, ethicists, legal and regulatory experts, and community members who are not directly engaged in the research under consideration.
- The updated oversight process provides more flexibility to adapt the Guidelines according to the oversight norms in each country.

Categories of Research

- **Category 1A**: exempt from review by a specialized scientific and ethics oversight process.
- **Category 1B**: also exempt from review but should be reported to a designated institutional entity or body in order to monitor the research in case any significant issues arise.
- **Category 2**: permissible after review and approval through the specialized scientific and ethics review process.
- **Category 3A**: prohibited despite scientific rationale, because it is currently unsafe.
- **Category 3B**: no scientific justification, unsafe, and/or widely considered unethical.

The Guidelines specify types of scientific projects that should be subject to specialized review and the level of review.

Categories of Research

CATEGORY 1	CATEGORY 2	CATEGORY 3
1A – Exempt from review by a specialized oversight process - Most *in vitro* pluripotent stem cell research - Most *in vitro* organoid research - Transfer of human stem cells into postnatal animal hosts	**2 – Reviewed by a specialized oversight process** - Procurement of embryos, or gametes for the creation of embryos, for *in vitro* research - Derivation of cell lines from human embryos - Genetic alteration of embryos or gametes - *In vitro* culture of human embryos for research for up to 14 days - Human cells transplanted into nonhuman embryos that are gestated in a non-human uterus - Integrated stem cell-based embryo models - Transferring human embryos following MRT into a human uterus	**3A – Not allowed: currently unsafe** - Germline genome editing - Transferring mtDNA-modified (not including MRT) embryos into a uterus - Using gametes differentiated from human stem cells for reproduction
1B – Reportable, but not typically reviewed by a specialized oversight process - Non-integrated stem cell-based embryo models - *In vitro* culture of chimeric embryos (human cells into non-human embryos) - *In vitro* gametogenesis without fertilization or generation of embryos		**3B – Not allowed: lacks compelling scientific rationale and/or is ethically concerning** - Gestating human stem cell-based embryo models - Human reproductive cloning - Breeding human-animal chimeras where there may be human germ cells. - Transferring human-animal chimeric embryo(s) to a human or ape uterus - Transferring human embryo(s), irrespective of origins, to an animal uterus

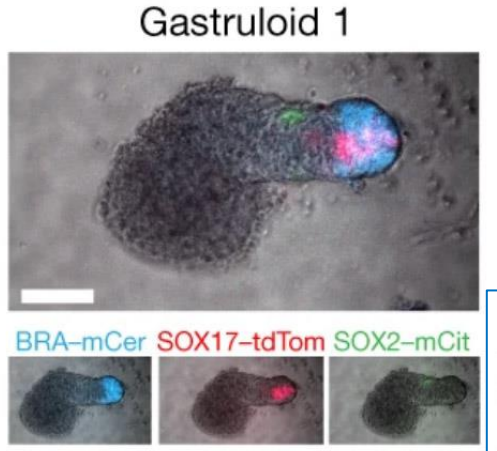
Culture of Human Embryos

Category 2 (subject to specialized review)

- Moves culture of human embryos beyond the 14-day limit from Category 3 (prohibited) to category 2, with a robust process to review any such experiments, and with a ….
- …. New recommendation to encourage public dialogue:

  ….. *national academies of science, academic societies, funders, and regulators to lead public conversations touching on the scientific significance as well as the societal and ethical issues raised by allowing such research. Should broad public support be achieved within a jurisdiction, and if local policies and regulations permit, a specialized scientific and ethical oversight process could weigh whether the scientific objectives necessitate and justify the time in culture beyond 14 days, including whether there are no suitable alternatives, and ensuring that only a minimal number of embryos are used to achieve the research objectives.*

Early Embryo Models



Moris, et al, Martinez Arias *Nature*, June 2020

Category 1B (reportable; exempt from specialized review)

- Models that experimentally recapitulate some, but not all aspects of the early post-implantation embryo.
- Gastruloids are an example of a **non-integrated stem cell model**.

Category 2 (subject to specialized review)

- Models that contain a range of embryonic and extra-embryonic structures and could potentially achieve the complexity to undergo events of early organogenesis and placental formation *in vitro*.
- Maintained in culture for the minimum time necessary to achieve the scientific objective.
- Blastoids are an example of an **integrated stem cell-based model**.

Category 3B (prohibited)

- Transfer of human stem cell-based embryo models to a human or animal uterus.

Chimeras and Chimeric Embryos

Category 1A

- **The transplantation of human stem cells, their derivatives, or other human cells into non-embryonic animal hosts.**

Category 1B

- **Transfer of human stem cells into non-human embryos and cultured *in vitro.***

Category 2

- **Transfer of human stem cells into non-human embryos *in vitro* followed by transfer to non-human uterus, excluding great and lesser apes.**

Category 3B

- **Animal chimeras incorporating human cells with the potential to form human gametes are bred to each other.**

Category 2 – New Recommendation

…. [chimeric embryo research] must proceed incrementally, stopping at well-defined timepoints to assess the degree and scope of chimerism during development before proceeding to full gestation, if full gestation is among the well-justified goals of the research.

To avoid unpredictable and widespread chimerism, researchers should endeavor to use targeted chimerism strategies to limit chimerism to a particular organ system or region of the gestating chimeric animal.

Reasons for going beyond 14 days:

- Basic knowledge of human embryology and our beginnings – what makes us human ?
- Knowledge relevant to potentially reduced miscarriage, congenital defects or even clinical problems arising later in life (Barker hypothesis)
- Important research on safety and efficacy that could be done on human embryos post-14 days
- Many of the methods around assisted conception (IVF) are still rather experimental. Tests are routinely only done on a few animal embryos and perhaps some human embryos up to blastocyst stages.
- There are a number of potentially new methods that need better testing than permitted by embryo culture up to 13 days.
  - Mitochondrial Replacement Techniques
  - In vitro-derived gametes
  - Heritable genome editing

  It would be important to be able to examine what happens to tissues post-gastrulation (both extraembryonic and embryonic)

Conclusions

- It is possible to make a strong scientific case for permitting culture of human embryos beyond 14 days, with appropriate regulation, ethical review, oversight, and after public views have been sought.
- Knowledge gained could help couples undergoing IVF, reduce rates of miscarriage and congenital defects, and allow better safety testing of new IVF-related procedures.
- Those affected by the above would need to support, and be involved in supporting, any change in the law.
- There needs to be some consistency with how stem cell-based embryo models are regulated – this will be important to give reassurance to both the public and scientists wishing to do the work. After all, many of the aims will be the same and the models are only valid if they mimic processes occurring in normal embryos.