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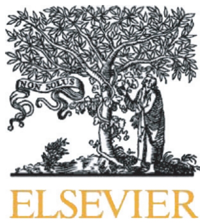

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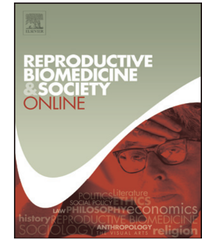

ORIGINAL ARTICLE

Adapting the 14-day rule for embryo research to encompass evolving technologies

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Abstract We consider the scientific evidence that research on in-vitro development of embryos beyond 14 days is necessary. We then examine potential new developments in the use of stem cells to make embryoids or synthetic human entities with embryo-like features, and consider whether they also require legal control. Next, we consider the arguments advanced against extending the 14-day period during which research on human embryos is currently permitted, and find none of them to be convincing. We end by proposing a new objective limit that could serve as a mechanism for regulating the use of embryos for research *in vitro*.

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The 14-day rule, proposed in the UK in the Warnock Report (1984), and then enshrined in law in the Human Fertilisation and Embryology (HFE) Acts of 1990 and 2008, is a limit that prevents the in-vitro culture of human embryos beyond 14 days after onset of embryo creation. It was developed in response to the introduction of in-vitro fertilization (IVF) in 1978, when the ability to develop embryos *in vitro* for research and for assisted reproductive therapy was demonstrated (Steptoe and Edwards, 1978). It is regulated in the UK by the Human Fertilisation and Embryology Authority (HFEA). In the USA, the 14-day rule has been followed since 1979, and it is now followed by law in at least 12 countries, with five other countries following this rule under national scientific guidelines (Hyun et al., 2016). It is one of the most internationally accepted rules in reproductive medicine (Appleby and Bredenoord, 2018). The placing of the boundary at 14 days originated because the primitive streak appears on the 15th day of human embryo

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development, as an objective visible streak of cells, at the onset of gastrulation (a period of cell internalization which forms the three germ layers – endoderm, ectoderm and mesoderm – as well as the body form and axes). The occurrence of gastrulation also signifies that an 'individual' embryo is now evident that can no longer split to form twins/triplets. The original HFE Act prohibited 'using an embryo for research purposes after the appearance of the primitive streak', which is deemed to be not later than 14 days since the day of gamete mixing (HFE Act, 1990). This was changed in 2008 to become: no culture beyond 14 days since 'the process of creating the embryo began', and applies to all 'live human embryos regardless of the manner of their creation, and to all live human gametes', thus updating the HFE Act to account for new technologies (e.g. certain cloning techniques) and to permit the regulated creation of 'admixed' embryos (a combination of human and animal genetic material) for research purposes (HFE Act, 2008). This addition to the 2008 Act shows that successful adaptation to follow scientific advances is possible. The 2008 Act also clearly delineated those embryos that could be used for research purposes – 'non-permitted embryos' (with which we are concerned in this paper) – from those that could be used in treatment – 'permitted embryos'.

When the HFE Act was developed, 14 days seemed plenty of time as embryo culture *in vitro* was limited. However, scientific advances have now taken us to a stage where the 14-day rule is seen as too restrictive. Here, we argue that the limit needs to be changed, based on ongoing embryological research, and propose how a new limit should be positioned.

What has been discovered to date in human embryo research?

Many discoveries have been made within the 14-day culture limitation, most being within the 7-day preimplantation period. Piliszek et al. (2016) described how the majority of transcription factors for lineage specification in mouse and human are the same; they just tend to differ in their timing, role and localization. In this way, humans differ from mice in their response to activation or inhibition of fibroblastic growth factor (FGF) signalling, which plays crucial roles in epiblast/hypoblast lineage specification in mice (Kuijk et al., 2012). One of the receptors for FGF (FGFR2) was not present in human blastocysts at day 6 (Kunath et al., 2014) and the hypoblast formed normally without FGFR, as did the epiblast and trophoblast. Also, in humans, the transcription factor CDX2 does not appear to be involved in trophectoderm specification at the morula stage, as it is only detected in outer nuclei from blastocyst expansion (Chen et al., 2009), in contrast to mice. OCT4 in humans became restricted to the inner cell mass alone at day 6 (was also present in trophectoderm at day 4), compared with day 3.5 in mice, so mutual CDX2-OCT4 inhibition does not appear to be necessary for inner cell mass/trophectoderm lineage restriction in the human (Chen et al., 2009).

Another example of a key difference between mouse and human embryo development is the timing of zygotic gene expression. Xue et al. (2013) used recent advances in single cell RNA-seq technology to investigate the regulation of genes in early mouse and human embryos. They found that both mice and humans exhibit a minor wave of transcription during the first cell cycle before cleavage, followed by a second major wave. Human embryos at the one-cell stage, when compared with mature oocytes, showed 149 differentially expressed genes. Similarly, mouse zygote pronuclei showed 520 transcripts upregulated in comparison with mature oocytes. This shows that both mammals have a conserved minor wave of ZGA prior to a major wave, although it is more pronounced in mice. In humans, it is thought that the major wave, involving large reprogramming of gene expression, occurs at the four-to-eight-cell stage on day 3 post fertilization (Braude et al., 1988; Niakan et al., 2012; Vassena et al., 2011), whereas in mice, it occurs at the second cell cycle, approximately 26–29 h post fertilization (Bolton et al., 1984; Hamatani et al., 2004; Vassena et al., 2011).

However, two main advances have been made recently, showing that embryos are more self-sufficient than was previously believed. In 2016, two groups reported successful in-vitro human embryo growth to 13 days (just before gastrulation), past the original hurdle of 7 days (implantation), showing they had created a maternal environment *in vitro* (Deglincerti et al., 2016; Shahbazi et al., 2016). This meant that gastrulation could potentially be analysed. This achievement came after a breakthrough in 2014 when it was discovered that human embryonic stem cells (hESCs) can self-organize into structures resembling post-implantation embryos ('embryoids'; Warmflash et al., 2014).

In 2014, Bedzhov and Zernicka-Goetz cultured mouse embryos *in vitro* after implantation, highlighting that the epiblast's self-organization into rosettes (with lumenogenesis forming the pro-amniotic cavity) was due to integrin/laminin signalling, not apoptosis. This research revealed dynamic interactions between the three tissues (pluripotent epiblast, primitive endoderm or hypoblast, and trophoblast) in the mouse to establish the body axis and form (Nuffield, 2017). Encouraged by this, Zernicka-Goetz's team attempted adaptations to their technique in order to culture human embryos, using unwanted embryos from IVF clinics. Mouse culture was difficult, so human culture seemed impossible, and in fact, most earlier knowledge came from monkey embryo culture experiments (Enders et al., 1986; Shahbazi et al., 2016). Brivanlou is reported as noting that the mouse studies had left them ill-prepared to culture human embryos after implantation, stating that '[the mouse embryo] should no longer be qualified as the closest model to humans' (Morber, 2017). Thus, although mouse and human pre-implantation embryos develop similarly morphologically, they do so at different rates, with mouse blastocysts forming at 3 days and implanting at day 4–4.5, compared with days 5 and 7, respectively, in humans (De Paepe et al., 2014). In mice, an amnion forms at gastrulation, whereas this occurs just after implantation in humans, and in mice, the hypoblast/epiblast lineage segregation occurs prior to implantation, whereas this occurs after implantation in humans (Bedzhov et al., 2014). Moreover, the two species display different molecular underpinnings (see above) and differ structurally after implantation, with the mouse epiblast becoming a cylindrical shape and the human epiblast becoming a flattened disc (Shahbazi and Zernicka-Goetz, 2018). All of these considerations illustrate

why it is essential to use human embryos or 'embryoids' for research to understand human development and establish clinical benefits.

The human methodology was achieved by culturing fresh or frozen-thawed day 5–6 embryos, after removal of the zona pellucida with acidic Tyrode's solution, on optical-grade plastic microplates (for time-lapse confocal microscopy) with two changes of medium, and using 21% oxygen rather than the commonly used IVF condition of 5% oxygen (Deglincerti et al., 2016; Shahbazi et al., 2016). Five specific criteria indicative of a normal development pathway (in part, by comparison with the Carnegie stages of in-vivo developing embryos; Hertig et al., 1956) were set:

- Segregation of the epiblast and hypoblast within the inner cell mass of the blastocyst, and morphogenetic movements to form the bilaminar disc. The epiblast is signified by increased levels of OCT4 and Nanog, the pluripotency transcription factors, and the hypoblast is determined by increased GATA6 and Sox17 (hypoblast-specific transcription factors).
- Formation of the pro-amniotic cavity within the pluripotent epiblast cells of the embryonic lineage (via atypical protein kinase C localizing apically causing apical polarity in epiblast cells and lumen formation).
- This should have the effect of separating the pluripotent cells into the epiblast disc (embryo proper) and amniotic epithelium.
- Formation of the primary yolk sac within the hypoblast.
- Differentiation of the trophoblast to yield its characteristic two cell types – the mononucleated cytotrophoblast and the multinucleated polyploid syncytiotrophoblast.

All of these criteria were achieved in 30% of embryos, establishing in-vitro embryos to the point of gastrulation (Shahbazi and Zernicka-Goetz, 2018), a striking achievement, with potential to increase this percentage with extension to the HFE Act. Recently, the successful culture of cynomolgus monkey embryos for up to 20 days *in vitro* beyond the gastrula stage has been reported (Niu et al., 2019), suggesting that such a period of culture for human embryos should also be feasible.

This advance in in-vitro culture systems has allowed the modelling of chromosomal instability and the investigation of how mosaicism and chromosomal defects can affect development in the peri-implantation period (Popovic et al., 2019). Understanding the effect of certain mosaicisms in biopsies taken at the blastocyst stage may help us to link future implications to the defects, thereby facilitating the selection process of IVF embryos to improve outcomes. Before this study, and before in-vitro culture beyond implantation, the ability of mosaic blastocysts to develop had only been investigated by studies on the mouse embryo (Bolton et al., 2016) or via clinical outcome data (Greco et al., 2015), with no blind studies assessing the IVF outcome of reported mosaicism. Without knowing the implications of a mosaic embryo for IVF transfers, it is difficult to determine whether to discard them and waste a potentially viable embryo or to transfer a potentially harmful embryo. With the UK National Health Service, and patients themselves, spending thousands of pounds on IVF cycles, and significant emotional turmoil after each failed attempt, this is a key issue. The study by Popovic et al. (2019) is thought to be the first to investigate the fate [via high-resolution next generation sequencing (NGS)] of chromosomal abnormalities and mosaicism in embryos up to 12 days post fertilization (dpf). Whilst the study showed that many mosaics at the 5/6-day blastocyst biopsy were no longer mosaic at 8 or 12 days, others were or had become uniformly aneuploid. In general, mosaic blastocysts diagnosed with a higher percentage of abnormal cells were more likely to be non-viable at 12 dpf. However, the study also showed that NGS can have an 18% misdiagnosis rate for mosaicism, so some improvement and further studies are necessary before this information can be applied in clinical scenarios. However, this study shows the developments possible from these advances in extended culture.

Scientific and clinical advances to be gained by extension beyond 14 days

Extension of the limit would allow in-vitro studies elucidating events of the 'black box' (days 7–28, where the embryo is implanted and thus impossible to investigate) by allowing the study of gastrulation; establishment of the neural plate and tube, major organs, and body axes; and the origin of primordial germ cells (PGCs) (Appleby and Bredenoord, 2018). Additionally, epithelial–mesenchymal transitions (EMTs) could be studied, unveiling how epithelial cells lose their organization (delaminate) and migrate as a mesenchymal cell in a disorganized cell layer, as well as morphological study of how the embryonic disc folds ventrally, to form a tube, and the role of the notochord in this process, as well as its role patterning the neural tube and somites.

This period also holds clear clinical benefits because days 14–28 are when embryological defects tend to occur. Organogenesis begins around 21 days and is when the embryo is most sensitive to teratogens, when heart and neural tube development begin. Defects in neural tube formation are among the most common serious congenital abnormalities. Additionally, before 4 weeks, the mother may be unaware of the pregnancy, making alcohol-/drug-induced defects much more likely (Nuffield, 2017). Almost 50% of all fertilized eggs die before the mother is aware she is pregnant, with 20–25% dying within the first 7 weeks (Morris, 2017; Nuffield, 2017). Additionally, 70% of IVF conceptions fail (Morber, 2017). Research on implantation, teratogens, miscarriages and birth defects could enhance pregnancy rates and IVF success by finding markers of incompetent embryos and using CRISPR-Cas9 as a research tool to elucidate gene function. As PGCs are specified around week 2.5, an extension to the HFE Act could aid understanding of germline cancers, and how PGCs and the early embryonic environment transfer epigenetic programming to future generations and affect adult health (Fleming et al., 2018). It would also facilitate clinical applications of stem cell research (regenerative medicine) by improving production of functionally differentiated cells.

Embryoids

The importance of understanding gastrulation in conjunction with restrictions imposed by the 14-day rule has led

researchers to find alternatives to in-vitro embryo culture, such as using hESCs to form embryoids [also known as 'synthetic human entities with embryo-like features' (SHEEFs)]. Embryoid experiments were first elucidated in mice. These experiments initially used ESC culture alone (Shahbazi and Zernicka-Goetz, 2018) but, when cultured, they did not show morphogenesis of the epiblast [e.g. polarization into a three-dimensional (3D) rosette structure that undergoes lumenogenesis], as early embryo development requires coordinated interactions of different cell types (Bedzhov and Zernicka-Goetz, 2014). Addition of a 3D extracellular matrix (based on work by Bissell and Mostov, e.g. Simian and Bissell, 2017) led cells to polarize and form a rosette, with vesicular exocytosis causing lumenogenesis after pluripotency exit, to form embryoids with a strong capacity to self-organize. In the mouse, there are also derived extraembryonic stem cell lines which can be used, such as the derived mouse trophoblast stem cell (TSC) line, which represents the extraembryonic ectoderm or trophoblastic stem cells. TSCs and ESCs cultured in a 3D matrix formed an embryoid that mimicked morphogenesis of post-implantation embryos, with a pro-amniotic cavity, symmetry breaking, and specification of the mesoderm and PGCs (Harrison et al., 2017). Most recently, complete embryo-like structures have been formed by adding extraembryonic endoderm or hypoblast (XEN) cells (which represent the stem cell population of the hypoblast, being more representative of parietal endoderm than visceral endoderm) to ESCs and TSCs, with all three tissues being formed: epiblast, hypoblast and trophoblast (Sozen et al., 2018). These structures undergo EMT at gastrulation, specify definitive endoderm-like cells and mesoderm cells, whilst also having a representative gene expression signature of the E7 mid gastrula embryo.

For humans, this line of experimentation has proved more difficult. For years, researchers found no human TSCs/XEN cells, so they had to develop a different approach. Like mice embryoids, they first cultured hESCs alone, which formed disorganized human embryoid bodies containing representative cell types of all three germ layers (Itskovitz-Eldor et al., 2000; Sharon et al., 2011), which can specify gastrula organizer-like cells. But there was no patterning resembling the structure of a human embryo, even if given appropriate signals. They took inspiration from studies of non-human primate ESCs (rhesus monkeys), where ESCs showed autonomous ability to form morphologically post-implantation embryoids *in vitro* (Behr et al., 2005). Therefore, hESCs were seeded on to circular micropatterns (plastic discs with patterned surfaces) to support clustering and self-organization of structures via confining space (Warmflash et al., 2014). When stem cells are confined to grow in circles, measuring a few hundred micrometres across, with addition of bone morphogenetic protein 4 (BMP4), they form radially symmetric patterns and concentric rings containing the three main cell types within 1–2 days, similar to those seen in gastrulating embryos. Morphologically, they did not look like real human embryos, but they did what was expected at a cellular and molecular level. The pattern is due to both exposure of BMP receptors and production of BMP inhibitor (Noggin). Exposure of these micropatterned cultures to activin-A/NODAL and WNT3A leads to what seemed to be a functional human 'organizer' (Martyn et al., 2018). It was previously undetermined if humans had 'organizer' cells, like other animals, to organize the formation of the head to tail axis and the nervous system. It is due to this synthetic hESC embryo advance that researchers have recently shown the existence of these organizers in humans, showing the promise of this line of research. Although this embryoid assembly lacks an amnion and amniotic cavity, this can be induced by adding 3D extracellular matrix (ECM), for example in the post-implantation amniotic sac embryoid (PASE) method of forming a human embryoid, instead of micropatterning (Shao et al., 2017). A PASE is a self-organizing cyst with an asymmetric epithelial pattern resembling an amniotic sac, formed using a gel bed of culture substrate and a 3D ECM environment overlay. These structures with ECM also resemble post-implantation embryos in other aspects, with spontaneous symmetry breaking and an amniotic squamous ectodermal epithelium adjacent to epiblast columnar epithelium, as well as gastrulation stages such as production of EMT transcription factors and mesoderm specification. These stages appear to be under the control of a BMP4 gradient, as in monkeys (Sasaki et al., 2016). Unfortunately, formation of the ECM culture PASE embryoids is less reproducible than the micropattern embryoids at present, so these strategies need to be developed. Recently, the culture of dissociated hESCs in a mix of liquid hydrogel/Matrigel has produced structures resembling day 10 epiblast (Simunovic et al., 2019) after 3 days; when cultured in intermediate concentrations of BMP4, these show breakage of antero-posterior symmetry and expression of markers of gastrulation. In light of these recent advances, we are starting to understand how cells self-organize and self-assemble, molecularly and cellularly, to generate discrete tissues and organs. This technology would also help us side-step the issue of a small supply of embryos for culture, and the potential ethical issues of embryo culture.

Bioprinting and other future developments

Additionally, there are new technologies on the horizon. Thus, derivation of human extraembryonic cell lines is underway. Human TSCs have been derived recently (Okae et al., 2018), allowing maintained culture of human villous cytotrophoblast cells. Additionally, Zernicka-Goetz's laboratory is attempting to derive hypoblast stem cell lines (like mouse XEN cells). Therefore, human ETX embryoids may be on the horizon, allowing us to replicate mouse protocols for human embryoids.

Another key developing technique is 3D bioprinting, in which different cell types and matrix are positioned precisely (Homan et al., 2016). Although functional organs have not been printed to date, organ tissue has been printed (Gao et al., 2017). Could this result in the printing of embryoids? Bioprinting is such a promising step in this field because it can manipulate cells and matrix deposition so that tissues can be constructed accurately. The required complex structure and architecture is entered into a computer aided file through 3D imaging, and printed in consecutive layers with predetermined X, Y and Z coordinates. Bioprinters may need to increase their resolution and speed to allow better interactions and regulations for

constructing entire 3D organs (Malkoc, 2018). The biomaterials and bioinks are a key limitation of this technology, and hESCs have seemed too fragile as a bioink to print in the past (adult stem cells or tissue cells are more predictable and easier to shape), but Scotland-based scientists at Heriot-Watt University and Roslin Cellab (Greenemeier, 2013) have reportedly developed a technique in which they place surviving cells in droplets of a regular size that go on to differentiate into different types of cells. They have developed a printing system that would not damage printed cells, driven by pneumatic pressure and controlled by a microvalve which opens and closes. Another issue is the vasculature of the 3D organ or embryoid. Diffusion alone can only function up to 150 μm thick, so solid organs need a vascular construct to be integrated at an early stage. A mechanism for this could be mixing bioink with angiogenic factors, but this is difficult to regulate. Alternatively, 3D printing can be used to print vasculature into 3D cell cultures, and this vasculature could be connected to an external perfusion apparatus (Kolesky et al., 2014). These advances are allowing for complex and structured SHEEFs that can recapitulate aspects of embryogenesis, and have the potential to develop progressively into more mature forms as they accumulate new embryonic features.

An ethical basis for extending the 14-day limit

Why does the law regulate in-vitro and in-vivo embryos so differently? Research embryos must be terminated at 14 days to prevent individuation, whereas in-vivo embryos can be terminated up to 24 weeks if continuance 'would involve risk, greater than if the pregnancy were terminated, of injury to the physical or mental health of the pregnant woman or any existing children' (Abortion Act, 1967). Objectively, more people will benefit from extended embryo research than from a termination, via establishment of therapies preventing congenital defects. It begs the question why embryo research is stopped so much earlier, especially when it is surely more ethical to use abandoned IVF embryos for beneficial research than to dispose of them. Additionally, the advance would help interventions to aid embryo survival in the long term. Clearly, different objectives are in play in these two scenarios, abortion being more about the rights of the woman to have control of her body whereas in-vitro embryos do not raise this issue. However, the points made above nonetheless seem to be pertinent. It could also be opined that the study of abortus material, whether induced or spontaneous from miscarriage, would be a better subject for study than embryos developing *in vitro*, an opinion we would not share due to both the rarity of such material and the inability to study it over time.

Various arguments against extending the 14-day rule have been advanced. One argument against changes to the rule is the fact that 14 days was selected due to its significance. At 14 days, individuation is said to commence, meaning that after this time, the embryo is no longer capable of forming twins/triplets but will develop into its own definitive person. However, not everyone agrees on the significance of this reasoning, and there is some disagreement on when the moral status of the embryo becomes prominent. Some believe it should be earlier (at fertilization), whilst others argue for it being much later, when the embryo becomes a fetus and can experience pain, have brain activity or even survive outside the womb. This argument for individuation is also less valid when we think about the fact that embryos used for research (not for transfer) would always be destroyed at 14 days (or at some limit) so have no prospect of being a person. How is the argument for gastrulation signifying individuality and increased potential for becoming a person valid if ultimately the research embryos never become a person? Additionally, against these arguments for keeping the rule as it is on moral grounds, we should discuss whether the rule was ever intended to be a moral status allocation. Many people believe it was set simply as a compromise to assign a space for research whilst showing respect for multiple views in a pluralistic society (Franklin, 2019). If this is true and the 14-day rule was not set for moral reasons alone, the cynicism regarding changing it is not entirely warranted, and a new legal limit that balances research advances and public trust can be developed to re-evaluate and keep the laws up to date with science, whilst being accepting of diverse views. In further argument, the committee of the Report Warnock (1984) was thought to never expect it to take over 30 years to maintain a live embryo in culture for 13 days, and therefore may have expected need for change earlier (Herbrand, 2016).

Additionally, Warnock has stated an objection to, and a warning against, changing the limit prematurely (Warnock, 2017). She was concerned that there is a risk that not only will the extension be denied, but we may risk the whole field of embryo research. She has stated that some people have little trust in scientists and their facts and arguments. Therefore, some politicians and media may take advantage of a new debate to encourage opposition to embryo research (potentially even including research prior to 14 days) or even to science and scientists in general, maybe to abortion and other issues regarding embryo protection. This is a large risk, and therefore a potentially strong argument for keeping quiet on the issue. She herself received accusatory and discrediting mail when she wrote the original Warnock Report (1984), so she appreciated the struggle of making monumental changes. She believed it is too soon to defend an extension when the limit has only just been reached, and felt that the science (and potential clinical application) was not yet sufficiently developed for there to be a persuasive argument to risk reopening the whole HFE Act at this time, and that the public needs more time to understand what researchers are doing and the implications of such research. For example, it took 6 years for the 1984 report recommendations to be absorbed into the 1990 HFE Act, so that the public had time to get used to the ideas within it (Johnson and Theodosiou, 2012). Warnock believed that scientists should spend more time researching days 7–14, so that discussions could occur about why further research is needed (Herbrand, 2016). However, a recent YouGov poll of the UK public, commissioned by the BBC and published in January 2017, although small (1740 people sampled), was promising for potential change to the limit: 48% were in favour of an extension to 28 days, 23% did not know, 19% wanted to keep the limit as it is and 10% wanted a complete ban on embryo research (Leida, 2017). This suggests that change is desired by half of the population (albeit a small sample size), but that more information is needed to equip the 23% who did

not know with the knowledge to make an informed decision. There needs to be a 'social consensus' on these complicated, delicate topics, so we should not advocate for change unless we are sure that there is no risk of major, effective resistance.

Additionally, with the UK playing a leading role in these debates, if we put forward a change in the limit, we are breaking a highly symbolic public understanding internationally, perhaps losing trust from other nations. Such a decision is likely to have a knock-on effect across the world, as the Warnock Report significantly guided and shaped the whole world's legal and ethical limits on such sensitive issues. A change from such a trusted and respected report may cause a lack of trust in science and could set a poor example to other nations, if we do not go about it in the correct manner. However, if the science requires such change, a considered argument will be presented. Arguments against change tend to focus on basic research and not clinical applications, so strong explanations of the tangible clinical benefits and what exactly scientists want to do are crucial. Additionally, on the other side of this coin, we could lose the international research community if we fail to adapt regulations, so we should not let fear of international public opinion dissuade scientific advances, and instead work to prove the worth of these advances.

A further argument is the infamous 'slippery slope', where it is suggested that after a change in the law, further change is far easier and we may never be able to keep firm control. People worry that this proposed change itself is an example of the beginning of the 'slippery slope', and that the boundary for research will keep extending as science develops. In refutation of this claim, we have regulatory bodies (HFEA) providing rigour to the system. Additionally, some technologies have been allowed that others disagree with for similar reasons, such as research on germline gene editing where oocytes/sperm/embryos can be genetically engineered (Nuffield, 2017). This change has not led to a descent into further legal changes. Additionally, as described above, the HFE Act has faced challenges and has managed to adapt successfully already. The limit can be moved. The ability to change a boundary does not imply there is a slope which is 'slippery' for us to descend down. The limit is a place where we can safely assess what lies on the other side before deciding whether to move or not. Also, some policies should be open to change, especially in controversial areas, as we ought to recognize that moral thinking, as well as science, evolves. For example, change in laws in favour of same-sex marriage and voting rights for women are seen as moral progress and not as slipping down a slippery slope. The 14-day rule is surely not the peak of ethical and scientific knowledge? We should not believe that we cannot develop our moral understanding and that science will not challenge our existing moral outlook. Therefore, we should be open to change, to address new possibilities, as a necessary part of ethical evidence-based policy. The use of the 'slippery slope' argument broadly reflects a lack of trust in science policy pathways and regulatory debates and implementations. If we were to make the consultation process transparent and informed by evidence, and engage with all views and opinions, we could increase trust to eliminate this argument (Cavaliere, 2017). Therefore, we need to engage with the public about why it is important, what scientists are doing and why they need to do this.

Another argument for keeping the law as it stands is based on the idea that the embryo may begin feeling pain and suffering after the primitive streak has developed. The Warnock Report (1984) stated that we should avoid research on humans with 'sentience' (Nuffield, 2017). However, at 28 days, there are no functional neural connections or sensory systems in the embryo (Hurlbut et al., 2017), so this is not a valid argument against the current period of research, or against change to the HFE Act up to 28 days. The first differentiated neurons with synaptic connections do not develop until late in the fifth week (34–36 days). Additionally, the first reflex arcs are not functional until the seventh week (49 days), and basic electroencephalogram (EEG) activity (fetal brain stem activity) is not discernible until about 7–8 weeks (Borkowski and Bernstine, 1955; Steinbock, 2017). Brain waves are necessary for sentience, but are not sufficient in themselves. Thus, any nervous system or potential sensory function are unlikely to exist until the seventh or eighth week at the earliest, making this argument not valid for an extension to 28 days or even a little further. However, this argument can serve as the basis for setting a new legal limit as we will now propose.

A proposal

What is clear is that, regardless of any extension, we need more clarity in how the HFE Act applies to emerging embryo research. It is unclear whether the HFE Act 2008 covers the stem cell method of creating embryoids: it does not specify embryos made by fertilization, but there is disagreement as to whether embryoids resemble embryos sufficiently to be classified legally as an 'embryo'. Likewise, it is undetermined how bioprinting will fit into current law. In future, bioprinting to form SHEEFs could potentially print an embryo that can develop through neurulation, but bypassing the primitive streak stage, by differentiation of hSCs into the three germ layers and patterning them with signals via 3D bioprinting into a synthetic model of an embryonic disc, and it is uncertain where this fits legally. Additionally, any form of revised boundary based on objective visualization of another feature or a certain developmental stage would not work in this realm either, as experiments could also be designed to bypass that stage (Aach et al., 2017). It might be helpful if the HFE Act could specify what was not an embryo, and therefore not covered by the Act.

The International Society for Stem Cell Research (ISSCR) released revised guidelines for stem cell research, proposing that the limit should include 'organized embryo-like cellular structure[s] with human organismal potential', and that these structures should be reviewed and prohibited if they breach the 14-day rule (ISSCR, 2016). However, defining 'human organismal potential' presents an issue known as the '14-day paradox' (Chan, 2018): we will not know if these embryoids have organismal potential without culturing them past the point when this potential would be obvious (14 days), so this ISSCR limit requires adaptation.

The Warnock Report (1984) stated that it is key to avoid research on embryos with 'sentience'. Thus, we propose the new guidelines should centre on this point, setting the new

limit at the stage prior to that at which the embryo/embryoid can perceive the environment and thus potentially feel pain, keeping this moral point at the heart of decisions. Although this would perhaps be difficult to enforce, this is necessary because a stage limit, such as gastrulation, cannot hold strong now bioprinting could bypass this stage. Additionally, Aach et al. (2017) argued that seemingly objective 'days since fertilization' limits present an insufficient limit for embryoids not formed by fertilization. Determining if pain can be felt is not obvious (Derbyshire, 1999), yet we need a strict, clear boundary to reassure the public that it is not up to self-judgement, but a more objective test (e.g. development of the limb buds, number of somites or length of the embryo) could be developed. It is agreed that sensations such as pain require a fully formed pathway from receptors and sensory neurons in the periphery to the spinal cord, through the thalamus and into the cerebral cortex (sensory regions) where an awareness of pain can be processed and felt (Derbyshire, 1999). However, with issues concerning morals and fetal respect, it is best to be completely safe. We cannot take chances on something like this. Therefore, the limit should be set as early as any relevant marker is found. Thus, markers that neural crest migration has occurred (Betters et al., 2010) could represent a good starting point (e.g. markers such as Sox9, Sox10 and p75NTR, expressed in the migratory neural crest immediately dorsal or adjacent to the neural tube). The neural crest cells form the peripheral nervous system, and without this, the embryo cannot sense pain or perceive the environment. However, this marker requires that the embryo culture be stopped and the embryo examined histochemically, which is undesirable. Thus, we propose that a readily visible marker of development should be used instead, such as is exemplified by Carnegie stage 12 at 29–31 dpf, namely the acquisition of an upper limb of length 0.26–0.34 mm, 25–28 pairs of somites or maximum embryo length of 5.1 mm (O'Rahilly and Muller, 2010). This stage, which also coincides with closure of both neuropores, seems to be a safer, earlier marking point than setting it at 8 weeks (Carnegie stage 23) when EEG activity (fetal brain stem activity) begins, suggesting some brain integration (Bergstrom and Bergstrom, 1963; Steinbock, 2017). Brain waves are necessary for sentience, but are not sufficient alone, and probably do not represent cortical activity until around 22–25 weeks (Burgess and Tawia, 1996). Thus, even at 8 weeks, sentience is unlikely. Indeed, it is not until 10 weeks that nocioreceptors first appear (RCOG, 2010), and they do not connect to the spinal cord until 19 weeks (Konstantinidou et al., 1995), from when needle punctures elicit fetal withdrawal and a stress response mediated through the brain stem (Gitau et al., 2004).

Conclusion

In conclusion, the technologies discussed above provide great potential to be beneficial not just in advancing scientific discovery but with true clinical benefit. We could enhance regenerative medicine and IVF, reduce miscarriage rates, and prevent birth defects. Although the HFE Act has been integral to embryo research to date, technologies have expanded vastly in recent years and regulation requires careful re-planning. We have proposed a specific marker of upper limb and somite development, coinciding with early stages of neural development, as providing a safe limit for extension with a wide margin of security. As Aach said: 'All great scientific advances have a way of exposing the imprecision of common concepts and forcing people to rethink them' (cited in Regalado, 2017). We believe that the time has come to rethink the 14-day rule, but we accept that articles such as this need to generate wide discussion before a change in legislation can be expected.

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