Background Document on the scientific importance of embryo research beyond 14 days

No. 2023/16Ae, The Hague, October 31, 2023

Background document to:
The 14-day rule in the Dutch Embryo Act
Nr. 2023/16e, The Hague, October 31, 2023

Health Council of the Netherlands

Table 1 Alternatives to research with classic embryos

Alternative	Description	Comparison with stage of embryonic development	Advantages compared to human embryos	Disadvantages compared to human embryos
Foetal tissue obtained from abortions	Women who undergo a scheduled abortion (for medical reasons or otherwise) can decide to donate foetal tissue for research.	Such foetal tissue is available in developmental stages from 2 to 22 weeks; however, in practice embryos can rarely be found in the amniotic sac before day 28 of embryonic development.	In this case, the embryos in question are embryos that remain from IVF treatments; all organs and structures are available from 4 to 22 weeks and can (partly) resume development in vitro (but only to a limited extent, since there is no circulation and the tissue obtained is often not intact), or cells can be obtained to be cultured as organoids; in the case of abortion on medical grounds, chromosomal abnormallities will be known.	Not sufficiently available until week 6 of pregnancy (i.e. 4 weeks of embryonic development); the tissue is often not intact (meaning it cannot be used to study the development of the embryo in its entirety); ad hoc access to tissue; of varying quality, also depending on the mother's health (e.g. in the case of alcohol, narcotics or medicine use during pregnancy).
Animal models	Embryos of mammals such as mice, rats or non-human primates and, to a lesser extent, other mammals (bovine animals) or other animal species (zebrafish, fruit fly, etc.).	All mammals have a morphologically comparable pre-implantation period, but there are significant differences in the timing of processes. During implantation and the formation of the body axes, developmental processes are highly species-specific. Organ formation is relatively similar among mammals, but there are some significant differences, for example in brain formation. Organ formation in humans is a more complex and lengthier process than in mice. Moreover, human organs undergo exponential growth during development.	Animal models are physiological models. Subject to a proper infrastructure and ethical approval, animal models are accessible and available (it is easy to plan pregnancies in test animals, in terms of numbers and pregnancy stages); in many cases the genetic background is known; genes can be modified so as to study their function, and it is possible to visualise gene products or structures by marking them.	Due to significant differences with human embryos, the knowledge gained has limited value for human beings (as it cannot always be extrapolated).

Alternative	Description	Comparison with stage of embryonic development	Advantages compared to human embryos	Disadvantages compared to human embryos
Cell cultures	2D stem cell models that recapitulate some aspects (specific cell types) of a human embryo, during develop-ment and/or maturity (such as bone marrow stem cells). Normally, cell cultures are limited to cell types that do not usually develop into a mature stage, unless obtained from mature cells. Cell cultures contain one or a few layers of cells.	The same as in the case of organoids, depending on the origin of the cells. A cell culture can show less complex stages, because the cells remain in a 2D layer. In cell cultures with pluripotent stem cells, the cells develop from the primitive streak/ gastrulation stage, sometimes even into mature cell types. In many cases the development stagnates at a foetal stage. This is due to the fact that little is known about the signals required to make cells develop to maturity. Unlike organoids, whose growth is inhibited when there is no blood circulation, cell cultures do not need circulation. In many cases, the cells can be induced to differentiate further by including them in a 3D culture with cells from a specific organ.	The same as in the case of organoids. The advantages are that a cell culture is even simpler to interpret, because it is less heterogeneous (fewer different cell types together) and has a sheet structure. 2D stem cell models are easy to reproduce, and are ideal for medicine testing and for simultaneous testing of multiple diseases. It is compelling to do research with 2D stem cell models on a cellular level, for example by repairing a gene mutation and immediately testing it for functionality, or for understanding how cell types react. Because 2D stem cell models allow simultaneous testing of many diseases and the cells can be meticulously followed while cultured, these models are eminently suitable for research at a cellular level.	2D stem cell models are not physiological (as cells often behave differently in isolation than as part of a tissue) and of low complexity (cells in isolation are often found on a hard substrate/plastic). In other words, they represent neither the organ of an embryo nor the embryo itself in its entirety.

Alternative	Description	Comparison with stage of embryonic development	Advantages compared to human embryos	Disadvantages compared to human embryos
Non-integrated ELS	3D or 2D stem cell models that recapitulate some, but not all aspects of the embryo. Organoids are a type of non-integrated ELS. Non-integrated ELS may contain all embryonic cell types, but do not contain all extraembryonic tissues corresponding to the developmental stage they represent. **Non-integrated ELS are unable to represent the integrated development of the entire embryo.**	Non-integrated ELS can correspond to structures associated with developmental stages between day 5 and day 28. Little is known about the extent to which non-integrated ELS differ from embryos, so extrapolation should be applied with caution. Moreover, as a precautionary measure scientists actually prevent non-integrated ELS from developing beyond a structure resembling a day-14 embryo, given the 14-day rule.	While non-integrated ELS are not intended to represent the integrated development of the entire embryo, they may be suitable for the study of specific processes that occur in the early stages of development. Non-integrated ELS are able to recapitulate some processes that take place in weeks 3 and 4. As in the case of cell cultures (2D) or organoids (3D), it is possible to use genetically modified stem cells. In addition, non-integrated ELS are small, relatively easy to create, and reproducible. ELS are genetically identical to the donor of the stem cells used to create them.	Little is known about the extent to which non-integrated ELS differ from embryos, so extrapolation should be applied with caution. Since non-integrated ELS are unable to represent the integrated development of the entire embryo, it remains unclear how studies with non-integrated ELS should be interpreted in light of studies using entire embryos.

Alternative	Description	Comparison with stage of embryonic development	Advantages compared to human embryos	Disadvantages compared to human embryos
Organoids	3D stem cell models that represent parts of the embryonic development, and/or part of mature organs/tissues. This can be attained by creating several organs or structures at the same time and culturing them in vitro (e.g. axioloids, which contain both a neural tube and somites). Organoids can be created from a single stem cell or from multiple cells (of the same type or otherwise). Depending on the cell type, the number of organs and the degree of self-organisation of an organoid, it can be regarded as a non-integrated ELS, assuming that ELS are structures that recapitulate some or all aspects of the embryo.	Organoids can be created from stem cells, or from embryonic or mature tissue. The origin of an organoid determines the stage it represents. Organoids can also be made from pluripotent stem cells and go through the development of specific organs or tissues from the primitive streak/ gastrulation stage (the first step is the differentiation of the germ layers) up to and including a structure that presents at least one specific function of the organ. In most cases, the cell types in organoids made from pluripotent stem cells do not differentiate into the mature stage (but protocols are improving).	As organoids only recapitulate a part of the body/embryo, analysing them is not as complex. When organoids are made from stem cells, it is easy to modify them genetically or to visualise specific gene products or structures by marking them (as in the case of animal models). Organoids are small, relatively easy to create and also easily reproducible. This makes them highly suitable for use as a model for research at the functional unit level or tissue level. For example, organoids are attractive models for medicine testing.	Many organoids are characterised by very limited functionality and low complexity. These models are able to recapitulate simple structures, and will never develop to the mature stage of the organ concerned (unless the cells are from a mature organism, as in the case of tumoids). Organoids are not physiological models. They lack many cell types, and the organisation of cells and tissues in an organoid is not the same as in the organ it represents. Many organoids have no circulation, no innervation (nerves), no immune system, etc. Many types of organoids are cystic structures, containing one or two cell layers and a few cell types. These structures tend to be very small (several millimetres) and have very limited possibilities to grow due to the absence of blood flow. Organoids never represent the integrated development of entire embryos, but only parts of an organ or organ system.

Alternative	Description	Comparison with stage of embryonic development	Advantages compared to human embryos	Disadvantages compared to human embryos
Integrated ELS	Embryos created by combining the requisite cell types, resulting in an embryo-like structure. **An integrated ELS can model the integrated development of an entire embryo, containing all embryonic and extraembryonic cell types.**	It is inherent in the process of creating integrated ELS that the resulting structures at their creation are comparable to embryos that are 5 to 10 days old. After that, such ELS can be further cultivated in the same way as classic embryos (that are formed by fertilisation). Since little is known about the extent to which ELS differ from embryos, it is important to exercise caution when extrapolating. Moreover, as a precautionary measure scientists actually prevent certain ELS from developing beyond a structure resembling a day-14 embryo, given the 14-day rule.	ELS are easier to produce, and can be created in larger quantities than embryos. This is because no donors of egg cells and sperm cells are needed, only donors of the stem cells to be used. Moreover, it is relatively easy to genetically modify ELS (even before the full structure is formed).	At present, ELS are expected to achieve the complexity to manifest the ability to undergo futher integrated development. However, it is not clear whether integrated ELS are 100% identical to classic embryos and to what extent they are capable of developing further. Genetically, integrated ELS are less diverse than classic embryos, because they are genetic clones of the cells from which they are created. According to the Health Council of the Netherlands, integrated ELS do not constitute a morally preferable alternative to the use of classic embryos, and are entitled to the same level of protection.

Literature

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Table 2 Developmental processes and related disorders in weeks 3 and 4 of embryonic development

Developmental period	Week 3 (day 15 – day 21 after fertilisation)	Week 4 (day 22 – day 28 after fertilisation)
What happens during this period?	Gastrulation (formation of the three germ layers); formation of blood islands and primitive haematopoiesis in the yolk sac; formation of the primordial germ cells, the neural plate, beating heart tube and onset of blood circulation	Start of morphogenesis of the heart (cardiac looping); neurulation; migration of neural crest cells (peripheral nervous system); formation of precursors of the intestines, lungs, liver, pancreas, kidneys, muscles, vertebrae, skin and (para)thyroid glands; definitive haematopoiesis (formation of blood cells and platelets); onset of limb formation, precursors of the eyes and ears; migration of primordial germ cells to the gonads and onset of development of the immune system
What could we learn from cultivating an entire embryo during this period?	The formation of the three body axes (dorsoventral, anterior-posterior and left-right); body segmentation; onset of the blood circulation and development of interaction between mother and foetus	Folding of the body; formation of the major body cavities; onset of organ patterning and regionalisation along the three axes; cell migration (neurons, germ line, blood cells and immune cells); initial stage of development of the precursors of all organs; growth of the placenta and the amnion
Which developmental disorders would we understand better?	Diseases associated with morphogenesis, which require the opportunity to study the embryo as a whole. Examples: conditions associated with abnormalities in the formation of the three body axes (cyclopia, situs inversus, caudal dysplasia); cardia bifida; twinning abnormalities (conjoined twins) and midline defects (holoprosencephaly)	Diseases associated with morphogenesis, which require the opportunity to study the embryo as a whole. Examples: body cavity wall defects [gastroschisis, omphalocele, Eagle-Barrett syndrome, bladder exstrophy, ectopia cordis (embryo folding fails)]; neurulation issues; midline defects; neural crest diseases, associated with the migration of the neural crest cells (e.g. Waardenburg syndrome, Hirschsprung's disease); scoliosis; teratomas (ectopic germ line tumours) and congenital defects of all organs
What alternatives are available to study the developmental processes during this period?	• Animal models • Organoids and other non-integrated ELS, such as gastruloids/axioloids can be used for research into (pattern) formation of the neural plate, somites and segmentation; heart organoids are suitable for research into the formation of cardiomyocytes • Cell cultures: for germ cell specification	• Animal models • Organ-specific organoids • Certain non-integrated ELS for research into the precursors of organs and regionalisation • Cell cultures that represent specific cell types

Developmental period	Week 3 (day 15 – day 21 after fertilisation)	Week 4 (day 22 – day 28 after fertilisation)
For which developmental processes are no alternatives available?	Formation of the body axes as a whole and in relation to the extraembryonic tissues; onset of the blood circulation; morphogenesis of the heart and neural plate (animal models are available to a limited extent)	Diseases that require the possibility to study a complete embryo, such as problems during migration of the neural crest cells or germ cells; migration of immune cells and blood cells; folding of the embryo; formation of body cavities and cavity walls; morphogenesis of organs (e.g. cardiac looping / septation)

Literature

1 Schoenwolf G, Bleyl S, Brauer P, Francis-West P. *Larsen's Human Embryology*. Elsevier; 2021.

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