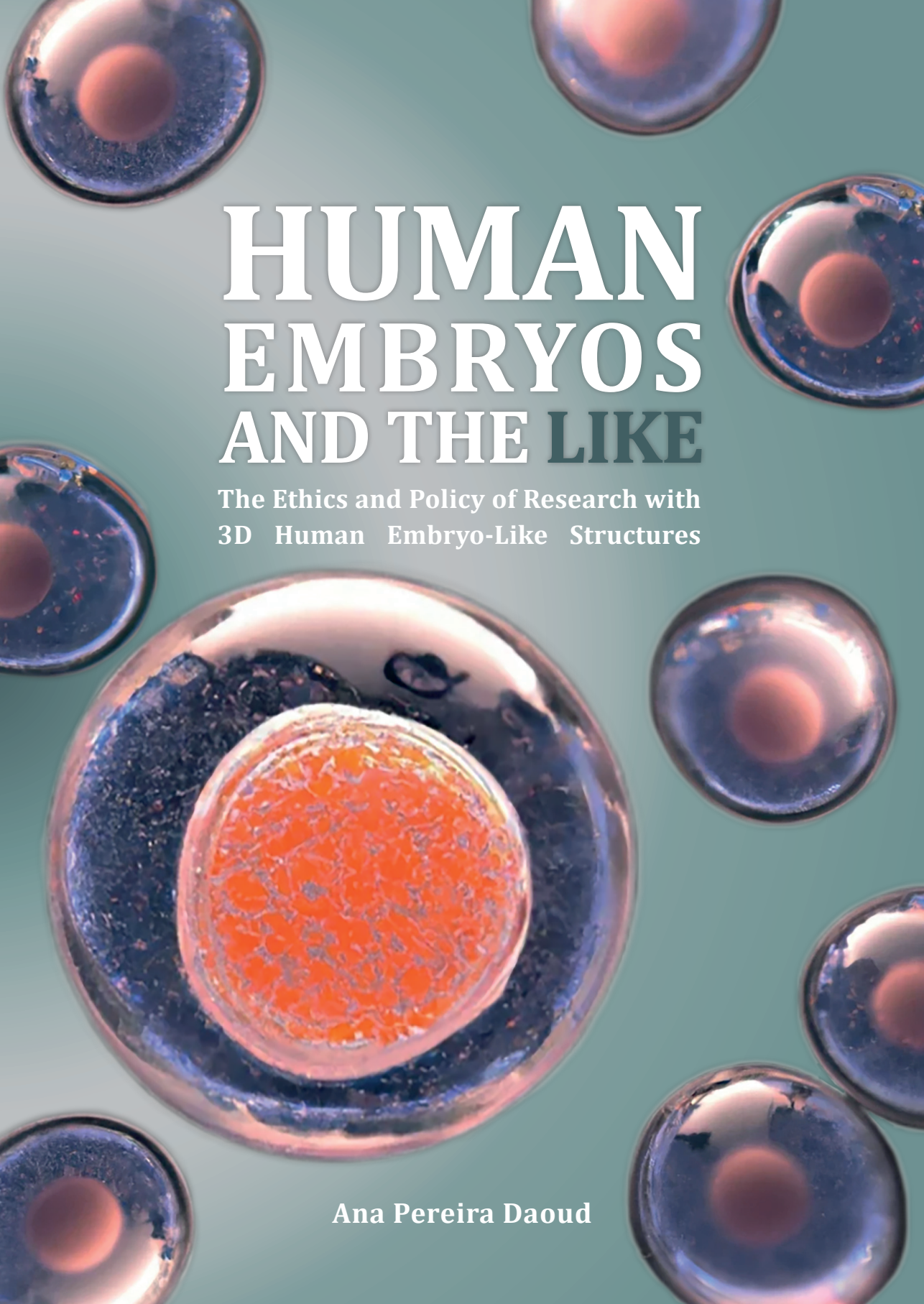
Human embryos and the like



HUMAN EMBRYOS AND THE LIKE

The Ethics and Policy of Research with 3D Human Embryo-Like Structures

Ana Pereira Daoud

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The Ethics and Policy of Research with 3D Human Embryo-Like Structures

DISSERTATION

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For past and future selves

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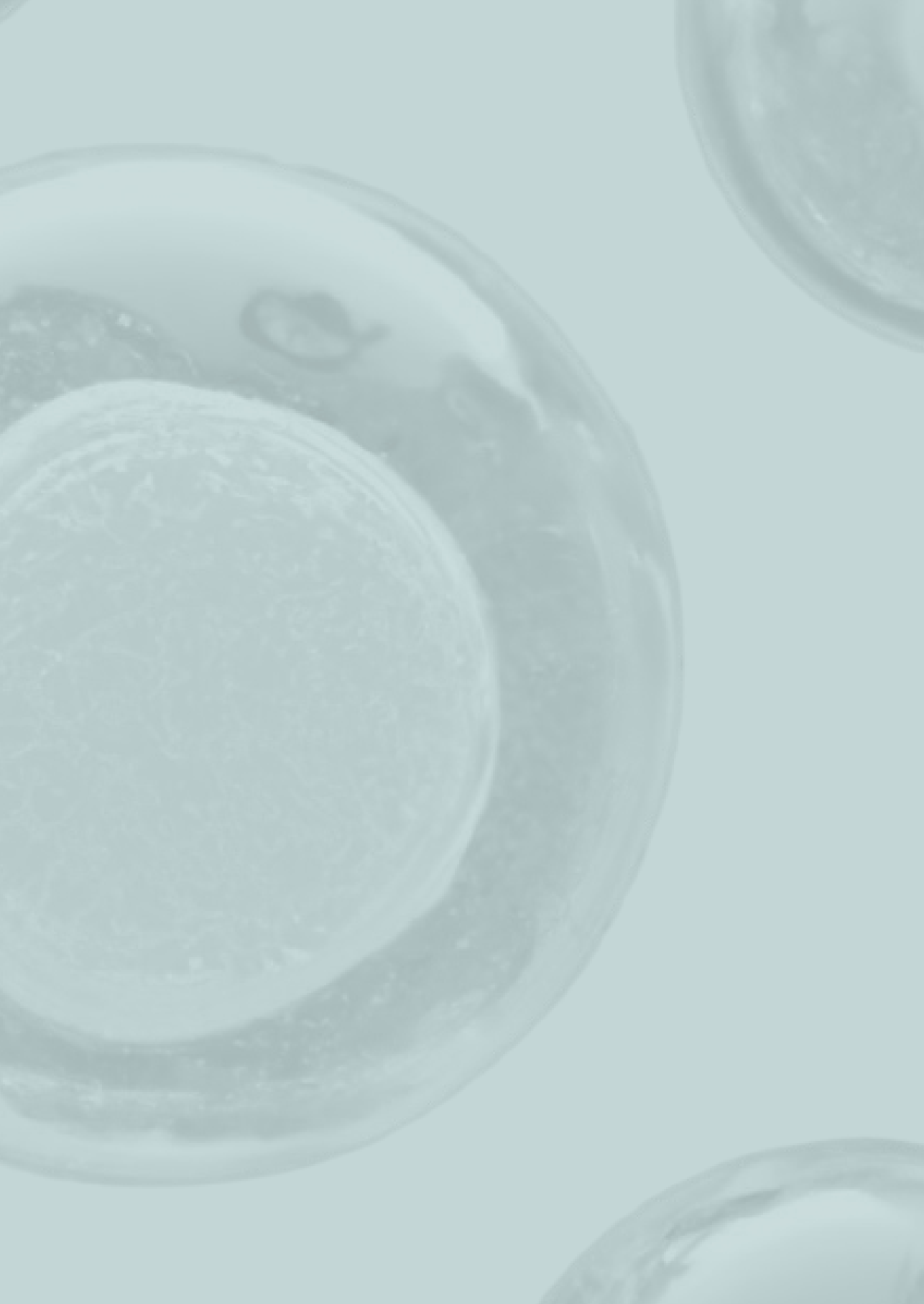

CHAPTER 1

General Introduction

GENERAL INTRODUCTION

My younger sister knew she wanted to have children before she even learned to speak properly. Her face would light up at the sight of a newborn; her baby dolls rocked to sleep as if they were made of flesh. Parenthood is often a matter of course. Our mother was the same growing up. She wanted to bear and nurture a child for as long as she could remember. Yet she lost at least three pregnancies before she could have us: two miscarriages in the first trimester, one stillbirth and, considering the overall high embryo mortality estimates before external signs of pregnancy can occur **(Jarvis 2016)**, possibly more. Parenthood is often also not a matter of course.

BACKGROUND

My mother eventually succeeded in having children without medical assistance, but many people today are still not as lucky as she was decades ago. In 2015, approximately 15% of couples globally were estimated to be affected by infertility **(Agarwal et al. 2015)**. In 2016, about four out of ten infertile couples seeking medical treatment were still not biological parents eight years after beginning medically assisted treatment **(Troude et al 2016)**. These numbers are striking, and they are only likely to increase as the prevalence of male and female infertility continues to grow **(Sun et al. 2019; Ravitsky & Kimmins 2019)**. Those that are fortunate enough to achieve parenthood through medical assistance often spend fortunes **(Katz, Nachtigall & Showstack 2002; Mladovsky & Sorenson 2010; Zhao et al. 2011)**, are rarely easily successful **(Zhao et al. 2011; Mantikou et al. 2013),** and face a higher risk of parenting children with congenital diseases **(Fauser et al. 2014; Pelkonen et al. 2014)**.

A more thorough understanding of early human embryogenesis can help comprehend and reduce the factors driving infertility, spontaneous abortions and congenital anomalies, but also the factors driving many genetic and developmental diseases that occur later in life but originate at early embryonic stages **(Gerrelli et al. 2015; Hyun et al. 2021; Adashi & Cohen 2022)**. Fundamental research into normal and abnormal development in human embryos can thus provide invaluable insights for many more fields of medical research than reproductive medicine alone. The use of human embryos (and later, human embryonic stem cells) has been the most meaningful way to gather these insights so far, but the recent development of stem cell-based models that are capable of mimicking embryonic morphology and functionality with increasing accuracy might provide similarly interesting alternatives. I will refer to this emerging field of biomedical research as human embryo modeling. Whether what makes human embryo modeling scientifically interesting would also make it ethically contentious is prompting debate, and that debate is the focus of this dissertation. In

the following sections, however, I will first take a step back and use my own mother's reproductive story as a thread to illustrate the relevant background.

Human Embryo Research in the 20th Century

When my mother experienced trouble carrying to term in 1979, Louise Joy Brown, the first child to have been born from *in vitro* fertilization (IVF) was just a few months old. The news of her birth, now coined one of the greatest breakthroughs of the 20th century **(Adashi & Jones 2012)**, astonished the world. For the first time in human reproductive history, it was possible to achieve live birth through the transfer of extracorporeal embryos to the womb and in the years following this discovery, millions upon millions would have been born, and millions more would have become parents, who simply could not have been if it were not for IVF **(Fauser 2019)**.

IVF technology made infertility patients like my mother hopeful, as well as many scientists. Confident that their clinical and fundamental understanding of human reproduction and human health more broadly could benefit greatly from human embryo experimentation **(Mulkay 1997)**, scientists hoped that the clinical introduction of IVF would make human embryos more readily and reliably accessible for research than ever before. Problems in human development and reproduction that had so far remained unexplained or only explained derivatively from animal studies, which do not always translate (safely) to human **(Prabhakar 2012)** and have their own share of technical, ethical, and legal challenges **(Kolar 2006; Ferdowsian & Gluck 2015)**, could then be studied more effectively and efficiently. At the same time, however, human embryo experimentation also sparked fear **(Mulkay 1996)**. Conventional social mores dictated that human life, and human conception specifically, were sacred and not to be tampered with. The first phase of ethical debate **(Ethics Advisory Board 1979)** began therefore well before Patrick Steptoe and Robert Edwards, the scientists responsible for developing IVF, could introduce the technology to the clinic. At the time, controversy centered on the destructive human embryo research experiments that same breakthrough had required **(Brinsden & Brinsden 2009)** and the question of whether scientists could be trusted with such valuable research material. By 1977, a U.S. federal Ethics Advisory Board had therefore already been requested to consider the ethical acceptability of supporting "research involving the fertilization of a woman's egg (ovum) outside her body" **(Ethics Advisory Board 1979, 1)**, which, despite being evaluated positively under condition by 1979, was not acted on until many years later **(Walters 2001)**.

While moral objections, blank legal bans, lack of funding, and technical difficulties slowed down progress **(Niederberger et al. 2018)**, research went forward where and how it could. By 1981, these efforts had paved the way to the successful culture of embryonic stem (ES) cells from mouse embryos **(Cyranoski 2018)**, which would later enable the development of many other biomedical approaches and technologies,

"including drug development paradigms, directed differentiation to treat specific diseases, nuclear transfer protocols used in cloning, and the establishment of methodologies for the isolation of non-rodent ES cells" **(Downing and Battey 2004, 1169)**. In the meantime, more and more experts were being brought together to consider if and how similar procedures involving human embryos should progress.

In the United Kingdom, the now renowned Committee of Inquiry into Human Fertilisation and Embryology had just been tasked "to examine the social, ethical and legal implications of recent, and potential developments in the field of human assisted reproduction" **(Warnock 1984, iv)**, including those of cloning through nucleus substitution. The Committee's report, presented in 1984, recommended the British government to permit and fund human embryo research under conditions of proportionality and subsidiarity **(Warnock 1984)**. Most of these recommendations, such as giving precedence to research with 'spare' over 'specially created' human embryos and prohibiting their culture beyond fourteen days post-fertilization (the 14-day rule), would nearly all later be translated into the Human Fertilisation and Embryology Act of 1990 **(HEFA 1990)**, the first law in the world to regulate human embryo experimentation **(LaTourelle 2014)**. The Committee's evaluation was not as widely shared then as it is now, however**.** Opponents of human embryo research argued that the recommendations were too lenient; whereas advocates argued that they were too restrictive and distrustful **(Mulkay 1997)**. In part to seek ethical consensus and "take a judicious position on what he knew from experience would be the controversies likely to dog the scientific progress of IVF" **(Niederberger et al. 2018, 305)**, a handful of experts, led by Edwards, founded the European Society of Human Reproduction and Embryology (ESHRE) in the same year the Warnock's Report was published.

Similar committees were being established in neighboring countries. In the Netherlands, which had a coalition government with a strong (Protestant and Roman Catholic) religious component **(Dondorp & de Wert 2020)**, the Health Council had also presented an interim report in 1984, two years after being tasked with advising the government about IVF and donor insemination **(Gezondheidsraad 1984)**. This initial report was brief on the ethical dimension of the technology, but its recommendations still "laid the basis for the licensing system under which IVF in the Netherlands is still being offered, making it perhaps the only country in the world where medically assisted reproduction is not commercially available" **(Dondorp & de Wert 2020, 261)**. A more thorough report of the Dutch Health Council was published in 1986, but its initial stance on the acceptability of human embryo research (albeit more carefully formulated) had essentially remained the same: research with human embryos could be allowed under strict material and procedural conditions **(Gezondheidsraad 1986)**. The particular recommendations of the Dutch Health Council were similar to those of the British Warnock Committee, with the notable exception of prohibiting the special creation of human embryos for research purposes. In spite of this, the report was considered

too liberal and at odds with Christian values, which is one of the reasons why its recommendations would not be formalized by law until many years later **(Dondorp & de Wert 2020)**.

By the time the first Portuguese *in vitro* baby had been born, in 1986 **(Diário de Notícias 2016)**, IVF procedures had started to become more commonplace. Nevertheless, human embryo experimentation remained controversial and most human fertility problems, including those of my Portuguese mother, understudied. Having been told that her acute eagerness for motherhood was the most probable culprit of her spontaneous abortions, she settled for the doctor's advice to proceed with adoption instead. In 1987, the same year the Catholic Church would express its doctrinal opposition to human embryo experimentation **(Pope John Paul II 1987)**, a brother and sister, about the age her stillborn daughter would have been, moved from the local Catholic orphanage into her home.

She had finally become a mother, but accepting that she may never birth a child was still no easy feat. The emotional, social, and psychological burdens associated with infertility can be excruciating and far-reaching **(Whiteford & Gonzales 1995; Podolska & Bidzan 2011)** and, for my then clinically depressed mother, they were. Yet out of the 186 million individuals estimated to be affected by fertility problems globally **(Agarwal et al 2015; World Health Organization 2020)**, her story is actually one of the more fortunate ones. However late and unexpected, she ultimately succeeded in carrying out two naturally conceived pregnancies in 1994 and 2000, respectively.

While my mother would insist that the births of my sister and I were the most remarkable news of that demi-decade, the birth of Dolly, the first cloned mammal to be born through the process of Somatic Cell Nuclear Transfer (SCNT) in 1996, was the one to hit the headlines **(Sinclair 2021)**. The announcement of her birth, in February 1997, triggered political and ethical debate across the globe, and has since become a symbol for "the imagined hopes and hazards of biotechnology, including … the promise of stem cell biology and regenerative medicine; the cloning of humans, interventions in the human germline and associated dystopian visions of our reproductive futures" **(Greenfield 2021, F70)**. The stem cell debate gained yet another layer in 1998, when the successful isolation and culture of *human* embryonic stem cells was first reported **(Thomson et al. 1998)**. Not only was stem cell derivation destructive for the human embryo from which they were retrieved, but Dolly was now also proof-of-concept that biomedical technologies could be used for applications that were as fascinating as they were frightening: research and reproductive cloning had now become feasible practices in human **(Cyranoski 2018)**.

In between these events, in 1997, the first and only legally binding document "to promote the protection of human rights in the biomedical field at a transnational level" **(Adorno 2005, 1)**, the European Convention on Human Rights and Biomedicine (also known as the Oviedo Convention) was being opened for signature. The Convention,

which prohibits the creation of human embryos for research purposes **(Council of Europe 1997)**, had been in the making long before Dolly and human embryonic stem cells could be reported, and the version initially adopted by the Council of Europe therefore did not address issues relating to human cloning **(Alkorta, Berian & Rodríguez-Arias 2013)**. The ban on procedures "seeking to create a human being genetically identical to another human being, whether living or dead" **(Council of Europe 1998)**, which would also include banning research cloning, was added through an Additional Protocol in January 1998 **(Adorno 2005)**.

The Convention went into force a month before the new millennium, in December 1999, and it has so far been signed by thirty-six Member States and ratified by twenty-nine **(Council of Europe 2021)**. The gap in number of signatures and ratifications is again in part due to disagreements about the scope of human embryo research that the Convention allows **(Goffin et al. 2008)**. For countries that ratify the Convention, it presumably implies that research is restricted to donated 'surplus' human embryos only, even though workarounds through embryo definitions have been documented (e.g., Spain and Finland) **(Alkorta, Berian & Rodríguez-Arias 2013)**. The Convention's implications for the permissible scope of research have also led some member states to refuse signing it altogether, albeit for opposing motives. Whereas "some countries (e.g., Germany) have not signed the Convention because it was deemed too tolerant—in that it allows some types of embryo research—others (e.g., the United Kingdom) have refused to sign the Convention because it was considered too restrictive in that it does not give researchers enough freedom to do research with human embryonic stem cells" **(Alkorta, Berian & Rodríguez-Arias 2013, 153)**.

By the time my sister was born, in November 2000, the sum of events of the late 20th century had led to a paradigm shift in policy and biomedical approaches to human embryo research. Laws were beginning to be established across the globe **(Matthews & Moralí 2020)**, and scientists were beginning to redirect their efforts towards developing tools that could replace or minimize the use of human embryos in research. Those efforts paved the way to the subject matter of this dissertation, for which I will use the umbrella term 'embryo-like structures', or 'ELS', for short.

Human Embryo Research and the 'Like' in the 21st Century

There are many umbrella terms for ELS **(Matthews, Wagner & Warmflash 2021)**, including 'stembryos' **(Veenvliet et al. 2021)** and 'stem cell-based embryo models' **(ISSCR 2021)**, but all attempt to communicate two key messages about them. The first message is that ELS are developed from (different types of) pluripotent stem (PS) cells. In the 20th century, the PS cells available for research were predominantly of embryonic origin (ES cells). This changed in the first decade of the 21st century, when insights from SCNT on cellular reprogramming, combined with insights from ES cell research on the conditions for maintaining pluripotency, made it possible to induce somatic cells back

into a naïve state **(Omole and Fakoya 2018)**. These cells, known as 'induced pluripotent stem cells' (iPS cells), were cultured from mice in 2006 **(Takahashi & Yamanaka 2006)**, followed by human in 2007 **(Takahashi et al. 2007)**. While iPS cells are by no means free of technical and ethical challenges (for example, because their culture is inefficient and amounts to a form of cloning), they have the advantage of holding "great promise for personalized cell-based therapy, human disease modeling, and drug development and screening" **(Omole and Fakoya 2018, e4370)** while bypassing the need for research with human embryos and associated burdens.

The advancement of iPS cells was quickly followed by the discovery that (embryonic and/or induced) PS cells, when placed "in a defined three-dimensional (3D) environment *in vitro*" **(Corrò, Novellasdemunt & Li 2020, C151)**, could "form mini-clusters of cells that self-organize and differentiate into functional cell types, recapitulating the structure and function of an organ *in vivo*" **(Corrò, Novellasdemunt & Li 2020, C151)**. These clusters are known as 'organoids', or 'mini-organs', and researchers have already succeeded in creating many different types, among which cerebral, gastric, retinal, endometrial, liver, kidney, pancreatic, and lung organoids **(Corrò, Novellasdemunt & Li 2020)**. Organoids are useful for many biomedical assays, "such as drug development, disease modeling, and ultimately—although admittedly this is currently still in the realm of science fiction—may even lead to clinical transplantation" **(Lensink 2021, 10)**, but their utility in fundamental and clinical research is limited to studies concerned with organs or organ systems, at most.

ELS are similar to organoids in terms of how they come to be, but have the additional advantage of enabling studies into local and/or temporal tissue differentiation at higher (organismal) levels. This brings me to the second point contemporary terminology attempts to communicate, namely that ELS are specifically created to model the three-dimensional complexity of (early) embryos. The ELS developed so far are admittedly still a long way from doing that faithfully, especially in human. At present, most models are cultured from animal PS cells and all of them seem to be incomplete in some morphologically or functionally significant way. 'Gastruloids', for example, which have been cultured from mouse and human PS cells, generate the embryonic cell lineages required to model body-axis formation and other important aspects of gastrulation, with more improved versions in mouse even producing "beating heart-like structures or … the precursors of the brain" **(Hubrecht Institute 2022)**, but lack (most of) the extraembryonic tissues that would be required for implantation. Other models are more complete in the sense of possessing greater differentiation potential, but still not complete enough to be capable of undergoing continuous development. 'Blastoids', for example, which model the blastocyst at peri-implantation stages and which have also been cultured from mouse and human (induced and embryonic) PS cells, can differentiate into embryonic and extraembryonic tissues, but still degenerate at some point in culture.

Despite remaining shortcomings, ELS have come a long way since their first ever culture, which dates back to roughly five years ago. The most recent versions of mouse blastoids, for example, have recently been shown to be capable of implanting in an artificial womb and recapitulating stages up to what would have been half the gestation time in mice **(Tarazi et al. 2022)**. While subsequent chapters will elucidate differences between past and current three-dimensional ELS in greater detail, these advancements attest to the rapid pace at which the field of human embryo modeling is developing. An unprecedented advantage of the field is that it provides bottom-up approaches to human developmental biology, which is not possible in research with human embryos. That does not mean that all ELS are developed to model the full organismal complexity of human embryos: sometimes research requires simplification, and some models are scientifically useful precisely because of how they provide simplified and decoupled versions of embryonic development *in vivo*. At the same time, it is conceivable that some models might be improved to the point of becoming virtually indistinguishable from human embryos. On top of the aforementioned advantages that human embryo models could provide for developmental biology (i.e., bottom-up and decoupled approaches to clinical and fundamental research), a major factor driving their development presumably is that they can bypass many of the legal and ethical burdens associated with human embryo research. Unlike human embryos, which are scarce for practical and normative reasons, these models can be cultured *ad libitum* and steer presumably sufficiently clear from the issues that have traditionally raised ethical and legal sensitivities in human embryo research. Whether that is truly the case, is now prompting extensive moral debate.

AIMS AND SCOPE OF THIS THESIS

This doctoral thesis inquires whether, and under what conditions, research with three-dimensional (3D) human embryo-like structures can provide an ethically acceptable alternative to research with human embryos. The main objectives of this inquiry are (1) to advance the emerging debate on the ethics of human embryo models, and (2) to contribute to the development of sustainable normative frameworks for their use in research.

METHODOLOGY: WIDE REFLECTIVE EQUILIBRIUM

John Rawls (1971) originally developed the Wide Reflective Equilibrium (WRE) method in the context of political philosophy, but it has gained wider acceptance as a coherence justification method in applied ethics. WRE is described as a process

of establishing coherence in a triad of moral beliefs, "namely (a) a set of considered moral judgments, (b) a set of moral principles, and (c) a set of relevant (scientific and philosophical) background theories" **(Räikkä 2009, 51)**. WRE is the most widely used method in applied ethics because of how it provides a tool for integrating practice and theory: moral judgments are often empirically informed, and WRE enables the use of empirical data as inputs to the normative analysis **(Nichols, 2012; Doorn & Taebi 2018)**. This ability to incorporate public perspectives into moral reasoning is an especially important advantage of the method from the perspective of responsible innovation in emerging biomedical applications **(Doorn 2013; Doorn & Taebi 2018)**, and the reason empirical data is part of this research study. The fact that WRE does not give priority to specific ethical theories or particular judgments and intuitions emerging from practice sets the method further visibly apart from other, narrower justification methods in applied ethics. In alternative approaches, moral justification would either rely on empirically informed views without subjecting them to ethical analysis (which could allow biases to sneak into the debate), or on specific ethical theories (such as Utilitarianism or Deontology) that inevitably involve upholding certain 'foundational' beliefs (i.e., beliefs that are non-inferential and that are therefore thought to reflect universal truths **(Hasan & Fumerton 2022)**). Both alternatives would thus ultimately rule out certain considerations from analysis at the onset and this would not only undermine the explorative character of my inquiry, but also prevent the inclusion of broader principles and theories that are nonetheless pertinent in medical research. After all, given how much was at stake in the associated debate about human embryo research, it is possible that human embryo modeling could lead to equally large and conflicting stakes. Proactive and inclusive analysis of the broad set of the judgments, principles and theories associated with human embryo modeling is thus a prerequisite for sound and evidence-based moral reasoning, and the WRE method seems to provide the most promising way to achieve that end.

When this research study started, however, very little was known about either theory or practice. Since three-dimensional human embryo modelling only began to emerge in 2017, the normative components of the analysis had to be derived primarily from the legal and ethical frameworks of the related debate on human embryo research. This included review of relevant legal and policy documents on human embryo research in jurisdictions within and outside Europe, as well as the biological and ethical literature on research with early human embryos. When scholarly literature on specifically human embryo research modeling became available over time, these were immediately included. The empirical study conducted as part of this dissertation was designed to help inform and enrich this analysis and consisted of individual and focus group interviews. (For an overview of the full research sample per group type, see **Tables I-III.**)

Table I: Research Sample of Focus Groups with Lay Participants

TYPE	SEX	AGE	EDUCATIONAL LEVEL*
FG-Pilot (n=5)	2/5 male 3/5 female	3/5 = 20 ≤ 30 years old 0/5 = 30 ≤ 40 years old 0/5 = 40 ≤ 50 years old 1/5 = 50 ≤ 60 years old 1/5 ≥ 60 years old	2/5 ≤ MBO 2/5 = HBO 1/5 ≥ WO
FG-Lay1 (n=10)	6/10 male 4/10 female	3/10 = 20 ≤ 30 years old 2/10 = 30 ≤ 40 years old 1/10 = 40 ≤ 50 years old 1/10 = 50 ≤ 60 years old 3/10 ≥ 60 years old	6/10 ≤ MBO 2/10 = HBO 2/10 ≥ WO
FG-Lay2 (n=11)	5/11 male 6/11 female	1/11 = 20 ≤ 30 years old 4/11 = 30 ≤ 40 years old 2/11 = 40 ≤ 50 years old 0/11 = 50 ≤ 60 years old 4/11 ≥ 60 years old	5/11 ≤ MBO 2/11 = HBO 4/11 ≥ WO
TOTAL (n=26)	13/26 male 13/26 female	7/26 = 20 ≤ 30 years old 6/26 = 30 ≤ 40 years old 3/26 = 40 ≤ 50 years old 2/26 = 50 ≤ 60 years old 8/26 ≥ 60 years old	13/26 ≤ MBO 6/26 = HBO 7/26 ≥ WO

**Education in the Netherlands discerns between Middelbaar Beroepsonderwijs (MBO, secondary vocational education), Hoger Beroepsonderwijs (HBO, higher professional education), and Wetenschappelijk Onderwijs (WO, higher scientific education).*

Table II: Research Sample of Focus Group with Professional Participants

TYPE	SEX	AGE	EXPERTISE
FG-Professionals (n=7)	2/7 male 5/7 female	0/7 = 20 ≤ 30 years old 1/7 = 30 ≤ 40 years old 3/7 = 40 ≤ 50 years old 1/7 = 50 ≤ 60 years old 2/7 ≥ 60 years old	4/7 ethics 3/7 law

Table III: Research Sample of Individual Interviews

Individual Interviews (n=5)	4/5 male 1/5 female	4/5 religious (Catholicism; Protestantism; Judaism; Islam) 1/5 non-religious (Humanism)

The focus group interviews, which lasted an average of two hours and took place between August and September 2020, had the particular advantage of providing an opportunity for participants to interact and engage with one another, and therefore allowed for a more thorough and nuanced understanding of participants' intuitions, motivations, and disagreements. In a sense, this was a way to use WRE collectively by allowing participants the opportunity to discuss and highlight contradictions in each other's beliefs. Of the four focus group interviews (N = 33) conducted in total, three were with lay participants. The first focus group with laypersons served as a pilot and therefore consisted of fewer participants (FG-Pilot, n = 5). These participants

were acquaintances of the author and selected based on demographic characteristics (sex, age, and educational level) of the Dutch population. To ensure that the other two focus groups with laypersons (FG-Lay1, n = 10 and FG-Lay2, n = 11) also consisted of a representative sample of the Dutch population, participants in these groups were selected by a professional recruitment agency and received a small financial compensation (€50,-) for participating in our study. The fourth focus group interview was conducted with health law and health ethics professionals selected from the supervisory team network based on their familiarity with debates on the ethical, legal, and societal implications of (comparable) emerging biotechnologies.

Since perspectives on human embryo research are often strongly intertwined with (non-)religious worldviews, and assuming that this might also be true for human embryo modelling, five in-depth interviews were conducted with participants reasoning from prominent worldviews in the Netherlands to complement the focus group data where appropriate. These interviews, which lasted an average of one and a half hours, were conducted online between August 2020 and March 2021 with participants known to engage in related bioethical debates from a Catholic, Protestant, Jewish, Islamic, and Humanistic perspective, respectively. For consistency, both focus group and individual participants received the same invitation letter and the same set of semi-structured questions. All interviews were conducted in Dutch, audio recorded, transcribed verbatim, and pseudonymized for thematic analysis. Individual interviews were summarized, and the summaries approved by the respective interviewees. Data analysis was performed from bottom to top: key passages in the transcripts were first tentatively open-coded, then validated through a randomized sampling procedure, and consequently clustered in a mind map based on the questions to which they referred. The resulting clusters were assessed and adjusted using the constant comparative method of analysis until all members of the supervisory team could agree on higher order themes. The thematic analysis resulted in four themes, which are reported in two separate articles.

In WRE, judgments arising from theory (principles and background theories; levels b and c) and practice (morally relevant facts and intuitions; level a) are integrated through a process of moving back and forth between beliefs at different levels and adjusting those that do not fit well into the triad without giving priority to beliefs at any particular level **(Räikkä 2009; Doorn & Taebi 2018)**. In this thesis, I do this by identifying areas of common ground in and between theory and practice, and agenda setting remaining incoherencies and open questions. The ideal outcome of the WRE process is to reach an endpoint (or "reflective equilibrium") where the beliefs at the different levels not only fit together, but also support each other **(Nichols 2012; Doorn & Taebi 2018)**. The beliefs that fall within this equilibrium are considered ethically defensible. However, this does not mean that they are incontrovertible. Since all levels of belief can furthermore be revised, real life situations can challenge previous beliefs and

the coherence between them. A reflective equilibrium is therefore always a provisional product and, in practice, an ideal that we might only be able to approach to the best of our ability **(Nichols 2012; Doorn & Taebi 2018)**. Whether the provisional equilibrium I arrive at at the end of this dissertation comes more or less close to its ideal is for the readers to judge for themselves.

OUTLINE

The contents of this thesis are grouped into a three-part funnel. Part I sets the stage for inquiry through contextualization and casuistry, Part II refines it by probing the initial findings empirically, and Part III narrows the discussion down to common threads, relates them to the main research question, and reflects on what they might imply within a broader research context.

Part I: Agenda-Setting through Contextualization
The first part of this dissertation juxtaposes the science and ethics of research with (human) embryo-like structures with that of traditional human embryo research as comparative normative framework for agenda-setting purposes. The aim is to acquaint the reader with embryo-like structures, map out the challenges that might arise when these structures are cultured from human cells, and identify the issues in need of ethical inquiry for further analysis in subsequent parts of the dissertation. This part consists of two separate chapters. **Chapter 2** provides a thorough overview of the state-of-the-art at the onset of this thesis and tentatively infers the conceptual, ethical and policy challenges likely to arise when similar progress is postulated in human. At the time of writing Chapter 2, only few and decoupled embryo-like structures had been cultured from human cells. That changed shortly after publication, first with the culture of human gastruloids in 2020, and then with the culture of human blastoids in 2021. **Chapter 3** uses these advancements (and the advancement of human blastoids, specifically) as a workable and real-life case study to further illustrate and expand upon the issues set out in the foregoing chapter.

Part II: Refinement through Empirical Validation
The second part of this thesis probes the findings of Part I empirically. The aim is to validate and complement the hypotheses previously set out whilst simultaneously reducing the present-day gap in empirical studies on public perspectives towards human embryo modeling. The data collected from these interviews is grouped into four main themes, which I present and discuss in two separate chapters. **Chapter 4** is concerned with themes pertaining to the participants' confidence in (the regulation of) research with human embryo-like structures, and reports only on data collected in

focus group settings. More specifically, it provides an overview of different outlooks towards the field, followed by an account of the factors influencing them. **Chapter 5** is concerned with themes pertaining to the participants' conceptual and moral qualification of human embryo-like structures, and reports on data collected from both focus group and individual interviews. Here, I focus in particular on differences in semantic and moral approaches to human embryo-like structures, as well as on what these differences imply for the acceptability of their use in research.

Part III: Discussion and Reflection

Part III discusses and ties together the main insights of the foregoing parts. The aim is to reflect on central red threads of the dissertation in order to answer the research question outlined above. This part also consists of two chapters. **Chapter 6** focuses on obtaining a deeper understanding of a recurring theme throughout this thesis: the so-called 'Argument from Potential'. The chapter provides a taxonomy of the argument and considers its moral bearing in relation to human embryo-like structures. In the General Discussion, **Chapter 7**, the focus lies on fulfilling the central aims of this thesis by reflecting on the main findings, conclusions and limitations, answering the main research questions succinctly, and reflecting on the implications and questions that these findings evoke when placed in a broader scientific context.

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PART I

Agenda-Setting through Contextualization

CHAPTER 2

Modelling human embryogenesis: embryo-like structures spark ethical and policy debate

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ABSTRACT

BACKGROUND

Studying the human peri-implantation period remains hindered by the limited accessibility of the *in vivo* environment and scarcity of research material. As such, continuing efforts have been directed towards developing embryo-like structures (ELS) from pluripotent stem cells (PSCs) that recapitulate aspects of embryogenesis *in vitro*. While the creation of such models offers immense potential for studying fundamental processes in both pre- and early post-implantation development, it also proves ethically contentious due to wide-ranging views on the moral and legal reverence due to human embryos. Lack of clarity on how to qualify and regulate research with ELS thus presents a challenge in that it may either limit this new field of research without valid grounds or allow it to develop without policies that reflect justified ethical concerns.

OBJECTIVE AND RATIONALE

The aim of this article is to provide a comprehensive overview of the existing scientific approaches to generate ELS from mouse and human PSCs, as well as discuss future strategies towards innovation in the context of human development. Concurrently, we aim to set the agenda for the ethical and policy issues surrounding research on human ELS.

SEARCH METHODS

The PubMed database was used to search peer-reviewed articles and reviews using the following terms: 'stem cells', 'pluripotency', 'implantation', 'preimplantation', 'post-implantation', 'blastocyst', 'embryoid bodies', 'synthetic embryos', 'embryo models', 'self-assembly', 'human embryo-like structures', 'artificial embryos' in combination with other keywords related to the subject area. The PubMed and Web of Science databases were also used to systematically search publications on the ethics of ELS and human embryo research by using the aforementioned keywords in combination with 'ethics', 'law', 'regulation' and equivalent terms. All relevant publications until December 2019 were critically evaluated and discussed.

OUTCOMES

In vitro systems provide a promising way forward for uncovering early human development. Current platforms utilize PSCs in both two- and three-dimensional settings to mimic various early developmental stages, including epiblast, trophoblast and amniotic cavity formation, in addition to axis development and gastrulation. Nevertheless, much hinges on the term 'embryo-like'. Extension of traditional embryo frameworks to research with ELS reveals that (i) current embryo definitions require reconsideration, (ii) cellular convertibility challenges the attribution of moral standing on the basis of 'active

potentiality' and (iii) meaningful application of embryo protective directives will require rethinking of the 14-day culture limit and moral weight attributed to (non-)viability. Many conceptual and normative (dis)similarities between ELS and embryos thus remain to be thoroughly elucidated.

WIDER IMPLICATIONS

Modelling embryogenesis holds vast potential for both human developmental biology and understanding various etiologies associated with infertility. To date, ELS have been shown to recapitulate several aspects of peri-implantation development, but critically, cannot develop into a fetus. Yet, concurrent to scientific innovation, considering the extent to which the use of ELS may raise moral concerns typical of human embryo research remains paramount. This will be crucial for harnessing the potential of ELS as a valuable research tool, whilst remaining within a robust moral and legal framework of professionally acceptable practices.

KEY WORDS

embryogenesis, stem cells, embryonic stem cells, trophoblast stem cells, blastoids, gastruloids, ethics, embryo-like structures, embryoids, pluripotency.

INTRODUCTION

Hindered by the inaccessibility of the *in vivo* environment, scarcity of research material and inherent ethical and legal constraints, studying the peri-implantation period in human remains a daunting task. To elucidate the complexities of embryonic development, continued efforts have been directed towards generating models that recapitulate embryogenesis *in vitro*. Notably, pluripotent stem cell (PSC)-based embryo-like models have taken precedence in complementing *in vivo* studies, contributing to the newly emerging field of synthetic developmental biology **(Ebrahimkhani and Ebisuya 2019)**.

In contrast to *in vivo* or *in vitro* embryos resulting from the process of fertilization, the entities at issue here are formed through stem cell coaxing, providing an amendable tool for mimicking developmental processes. This also implies a second difference in comparison to fertilization-based embryos, namely that their genome is not individually unique, but rather represents a genetic clone of the stem cells and/or donor somatic cells of origin. The general term for these entities is still under discussion, varying between embryoids, synthetic or artificial embryos, and synthetic entities with human embryo-like features (SHEEFs). Since some of these terms have either already been used in different contexts or prematurely denote these entities as embryos, we herein propose referring to stem cell-based embryo models as 'embryo-like structures' (ELS) to avoid misconceptions.

The considerable plasticity for modelling embryogenesis is not only alluring for human developmental biology but also holds vast potential for improving clinical approaches in assisted reproductive technologies (ART). Nevertheless, the features that make these structures scientifically interesting, also give rise to ethical and regulatory issues. It thus remains to be established whether ELS in fact represent a morally preferable alternative to research with human embryos.

The overall aim of the present article is two-fold. We endeavour to (i) provide an overview of the scientific approaches to generate ELS in both mouse and human, while discussing future strategies towards innovation in the context of human development and (ii) set the agenda for the ethical and policy issues raised by the generation, culture and use of ELS in a research context. We do so by drawing on prominent conceptual and normative insights from the human embryo research debate and inferring what they may imply for (research with) ELS.

Embryonic development in mice and humans

Mammalian development begins with the formation of the zygote **(Fig. 1)**. During the first days of development, the zygote undergoes regular mitotic divisions, yet transcriptional activity relies almost exclusively on maternal messenger RNA. The embryo undergoes a major wave of embryonic genome activation at the two-cell stage

in mouse and at the 4- to 8-cell stage in human **(Vassena et al. 2011; Niakan and Eggan 2013)**, an essential process for directing further developmental programmes.

Compaction begins at the morula stage, at the late 8-cell stage (embryonic day (E)2.5) in mouse and 16-cell stage (day 4) in human **(Table IV, Fig. 1)**. The compacted morula then starts to absorb fluid, establishing the blastocoel cavity as the hydrostatic pressure increases. This culminates in the formation of the blastocyst. At this stage, the inner placed blastomeres form the inner cell mass (ICM) at one side of the cavity, while those at the periphery establish the trophectoderm (TE), a thin single-layered epithelium. At E4.5 in mouse, the ICM further segregates into pluripotent epiblast progenitor cells (eventually forming the embryo proper) and the hypoblast or primitive endoderm (PE) which predominately contributes to the yolk sac endoderm **(Fig. 1) (Wamaitha and Niakan 2018)**. While these lineage contributions are likely to also apply in the human context **(Yan et al. 2013; Petropoulos et al., 2016)**, this process is yet to be elucidated. Interestingly, *in vitro* culture itself may influence lineage segregation dynamics in human. Blastocyst morphology, including ICM and TE quality, was shown to be significantly reduced in *in vitro* cultured embryos, compared to *in vivo* counterparts **(Munné et al. 2020)**.

In both mouse and human, the epiblast, PE and TE lineages are each marked by the expression of specific genes **(Fig. 1)**. While some lineage-specific markers are conserved between mouse and human, many others show species-specific expression dynamics **(Fig. 1) (Roode et al. 2012; Niakan and Eggan 2013; Blakeley et al. 2015; Deglincerti et al. 2016b)**. For instance, while TFAP2C is enriched in the mouse TE, it is expressed at similar levels in both the TE and epiblast in human. CLDN10 and PLAC8 are highly expressed in the human TE and not expressed in mouse TE, while Eomes is enriched in the mouse TE and completely absent in human **(Blakeley et al. 2015)**. Additionally, Cdx2 expression is specific to TE cells in mouse, yet shows variable expression in human **(Niakan and Eggan 2013)**. Moreover, FOXA2 is specific for human PE, while it is not expressed in mouse blastocysts **(Fig. 1)**. These differences highlight the diverging regulatory mechanisms underlying human preimplantation development compared to mouse.

Preimplantation development takes about 5–6 days in human and 4.5 days in mice. At this time, the blastocyst begins to hatch out of the zona pellucida and is ultimately able to interact directly with the maternal endometrium *in vivo*. Mammalian implantation relies on the developmental synchrony between the decidualized endometrium and the TE, while the peri-implantation microenvironment further mediates blastocyst attachment and invasion **(Table IV)**. These processes are regulated by factors secreted from both the maternal uterus and the embryo **(Cha et al. 2012; Gellersen and Brosens 2014; Salamonsen et al. 2016)**. This intricate discourse is crucial for embryonic development and ultimately the successful initiation of pregnancy.

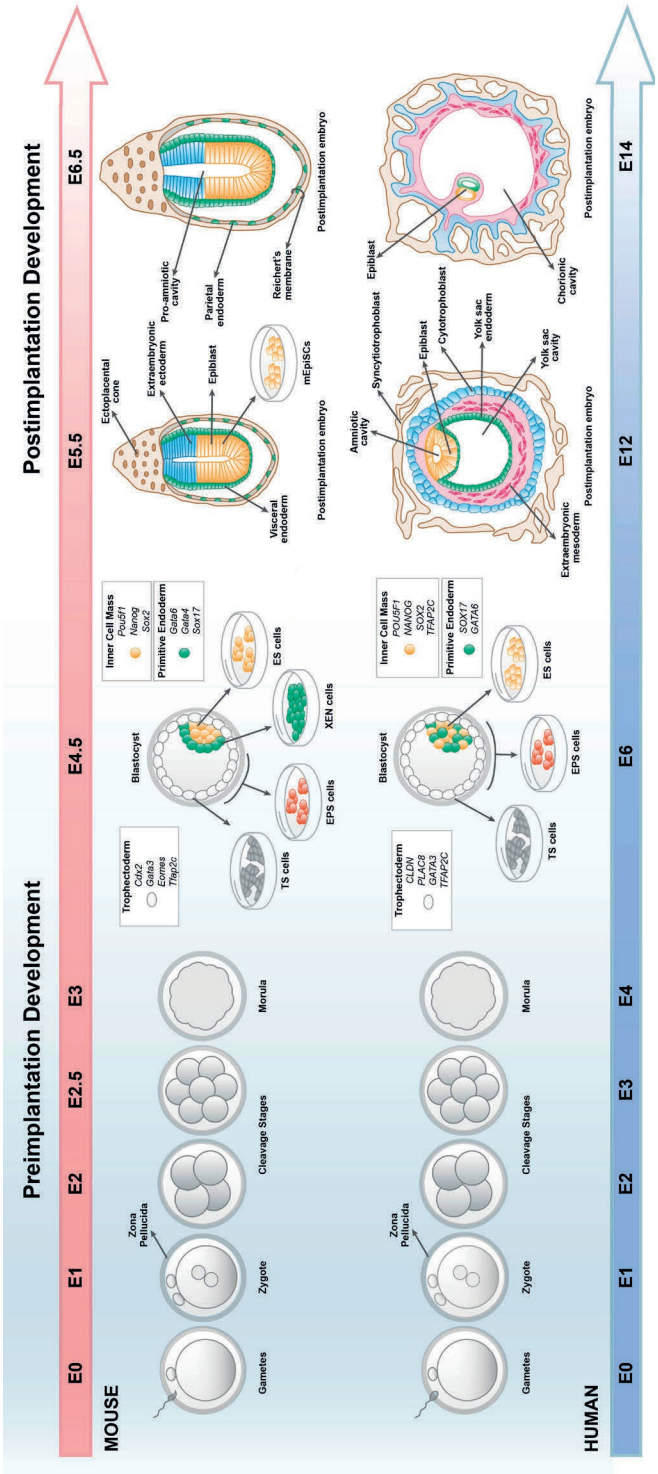

Figure 1: Overview of peri-implantation development in mouse and human. Lineage-specific markers of the trophectoderm, inner cell mass and primitive endoderm in both mouse and human are shown. EPS cells, extended pluripotent stem cells; ES cells, embryonic stem cells; mEpiSCs, mouse epiblast stem cells; TS cells, trophoblast stem cells; XEN cells, extraembryonic endoderm cells.

Following implantation, developmental progression appears to be less conserved between mouse and human **(Fig. 1)**. The mouse embryo elongates to form the characteristic egg-cylinder, with a cup-shaped epiblast, while in human, the epiblast remains flat or disc shaped **(Fig. 1)**. In mice, the polar TE is reshaped to form the extraembryonic ectoderm (which is absent in human) and the ectoplacental cone, which eventually contributes to the chorion and the placenta. In mice, both the extraembryonic ectoderm and the epiblast form cavities, which fuse into the pro-amniotic cavity **(Fig. 1)**. Specific to rodents, parietal endoderm differentiates and migrates from the PE and lines the TE, separated by a basement membrane. The structure is known as Reichert's membrane and functions as the parietal yolk sac, providing nutrients to the developing embryo **(Fig. 1)**. At this point, symmetry breaking starts and the distal tip of the visceral endoderm migrates to the prospective anterior part of the embryo, acting as a signalling centre to define the anteroposterior axis **(Takaoka et al. 2011)**. In humans, the amniotic cavity is immediately formed as the epiblast epithelializes and cavitates, early during implantation **(Fig. 1)**. Moreover, the extraembryonic mesoderm forms by day 12 post-fertilization in human, contributing to the chorionic cavity **(Dobreva et al. 2010) (Fig. 1)**. In contrast to humans, in mice, the amnion, yolk sac and chorion are only formed following the initiation of gastrulation **(De Sousa Lopes and Mummery 2014)**. In both humans and mice, the germline is set aside just before gastrulation, with the formation of the primordial germ cells **(Tang et al. 2016; Popovic et al. 2019)**.

Gastrulation serves as the gateway to shaping the body plan. During this process, the primitive streak (PS) coordinates extensive cell rearrangements that culminate in the formation of the three germ layers (endoderm, mesoderm and ectoderm) **(Table IV)**. Gastrulation is shortly followed by cardiogenesis, neurulation and initiation of somitogenesis **(Table IV)**.

Table IV: Table of definitions

SCIENTIFIC TERMINOLOGY	DEFINITION
Embryo-like structure(s) (ELS)	Umbrella term for pluripotent stem cell-based embryo models that resemble the morphology and physiology of fertilization-based embryos, as proposed by the authors.
Compaction	During compaction the originally round and loosely adherent cells of the embryo flatten, developing a polarity that maximizes contact between blastomeres. This reorganization involves the activity of cytoskeletal and cell adhesion elements.
Axis formation	The three germ layers (endoderm, mesoderm and ectoderm) are organized relative to a coordinate system (anterior-posterior, dorsal-ventral and left-right axes). This acts as a reference for the development and patterning of tissues and organs.
Gastrulation	In amniotes, gastrulation is the process by which the three definitive germ layers ectoderm, endoderm and mesoderm are formed from the epiblast. Each germ layer is lineage restricted and gives rise to specific organs.

Primitive Streak (PS)	The appearance of the PS marks the initiation of gastrulation. The PS is a transient structure that marks the posterior or caudal part of the longitudinal axis and bilateral symmetry in amniotes. The PS functions to channel epiblast cells fated to become (embryonic) mesoderm and (definitive) endoderm, which ingress to establish the germ layers. Cells of the epiblast at (or ingressing through) the primitive streak undergo an epithelial-to-mesenchymal transition.
Cardiogenesis	Cardiogenesis (or heart development) begins with the formation of the heart tube and the initiation of a heartbeat around 22 days post fertilization in humans and embryonic day 8.0-8.5 in mice.
Neurulation	Neurulation denotes the transition from the neural plate to the neural tube, the embryonic precursor of the brain and spinal cord. The closure of the neural tube (with closure of both cranial and caudal neuropore) is completed 26 days post fertilization in humans and at embryonic day 10.5 in mice.
Somitogenesis	Somitogenesis is the process by which two longitudinal rows of (paraxial) mesoderm flanking the neural groove condense into two strings of blocks called somites. The somites develop into (parts of) the skeleton, musculature and dermis. Somitogenesis takes place from around 21 days post fertilization in humans and embryonic day 8.0 in mice. Humans develop about 42-44 pairs of somites and this process is completed around 30 days post fertilization.
Twinning	Twinning generally stems from two situations during development. Dizygotic twins arise from the fertilization of two oocytes, which implant separately. In contrast, monozygotic twins are the results of a single fertilized oocyte splitting during early embryonic development. In monozygotic twinning: 1) if the splitting occurs during the cleavage stage, monozygotic twins will develop separate fetal membranes and placentas; 2) if the inner cell mass splits, twins will be surrounded by separate amnions, but share the same chorion/placenta; and 3) if the bilaminar disc splits, the twins will occupy the same amnion and share the same chorion/placenta. This last type of twinning can occur up to 14-15 days post fertilization.
Totipotency	Totipotency refers to the ability of a single cell to divide and generate all specialized cells of an organism, including embryonic and extraembryonic tissues. Accordingly, the zygote and individual cells of the cleavage stage embryo are the ultimate totipotent cells, as they can give rise to an entire organism.
Pluripotency	Pluripotency refers to the potential of a cell to generate embryonic or adult cell types, both *in vitro* and *in vivo*. The cells of the inner cell mass are pluripotent, as they maintain the potential to differentiate into any of the three germ layers: endoderm, mesoderm or ectoderm. Pluripotency can be captured *in vitro* through the derivation of embryonic stem cells or by direct reprogramming of somatic cells.
Multipotency	Multipotency refers to the potential of a cell to differentiate into a limited number of cell fates.
Somatic cell nuclear transfer (SCNT)	The developmental potential of somatic cells can be restored to totipotency by SCNT. SCNT is the process of transferring nuclear DNA from a donor somatic cell into a recipient enucleated mature oocyte, which can further give rise to an embryo. The SCNT-derived embryo can be used for the derivation of embryonic stem cells, which are genetically identical to the original somatic cell. This process is also referred to as therapeutic cloning.
Induced Pluripotent Stem Cells (iPSCs)	iPSCs are generated by reprogramming somatic cells into a pluripotent state similar to that of embryonic stem cells, by the forced overexpression of four transcription factors: Pou5f1, pou class 5 homeobox 1; Sox2, SRY-Box 2; Klf4, kruppel like factor 4 and c-Myc, c-myc. Induced PSCs overcome the need to use embryos for pluripotent stem cell derivation. Like SCNT-embryonic stem cells, iPSCs are genetically identical to the original somatic cell from which they are derived.
Embryonic Stem Cells (ESCs)	ESCs are pluripotent stem cells generally derived from the blastocyst inner cell mass.

Naive and primed pluripotency	Embryonic stem cells are known to exist in at least two states of pluripotency: naive and primed. Although derived from the same blastocyst stage inner cell mass, human and mouse embryonic stem cells show different properties reflecting these two states. Accordingly, mouse embryonic stem cells adopt the naive state, more similar to the preimplantation epiblast, while human embryonic stem cells are in a primed state, more akin to the post-implantation epiblast. Pluripotent stem cells in these two states show differing morphologies, distinct culture requirements and different transcriptional and epigenetic signatures. Swapping culture conditions can convert naive pluripotent stem cells to the primed state and vice-versa.
Extraembryonic endoderm (XEN) cells	XEN cells are multipotent stem cells that recapitulate properties of the extraembryonic visceral endoderm. To date, XEN cells have not been successfully derived from human embryos, however naive human embryonic stem cells have been shown to acquire characteristics of the extraembryonic endoderm in response to Wnt, Nodal and LIF signaling.
Trophoblast Stem Cells (TSCs)	TSCs are multipotent stem cells that recapitulate properties of the extraembryonic trophectoderm. In mouse, TSCs can be derived directly from blastocysts, but can also been induced (iTSCs) by reprogramming somatic cells using forced overexpression of four transcription factors: Tfap2c, transcription factor AP-2; Gata3, GATA-binding protein 3; Eomes, eomesodermin; and Ets2, E26 avian leukemia oncogene 2, 3' domain. In human, TSCs can be derived from blastocysts and first trimester placentas.
Extended pluripotent stem cells (EPS)	EPS are cells that exhibit totipotent-like developmental potential. They should harbor the capacity to contribute to both embryonic and extraembryonic lineages.
Embryoid Bodies (EBs)	EBs are three-dimensional aggregates differentiated from pluripotent stem cells with the purpose of obtaining cells of the three germ lineages *in vitro*.
Epithelial-to-mesenchymal transition (EMT)	EMT involves the transition of polarized epithelial cells towards motile apolar mesenchymal cells.
Decidualization	Decidualization of the human endometrium involves extensive morphological and functional differentiation of the endometrial stromal cells that begins approximately 6 days after ovulation. This process is critical as impairment results in implantation failure, recurrent miscarriage or pregnancy disorders, ultimately leading to infertility. Decidualized endometrial stromal cells provide nutrition to the implanting blastocyst and support further peri-implantation development.
Implantation	Implantation involves the attachment and invasion of the embryo within the maternal endometrium. The initial attachment phase encompasses apposition of the endometrial epithelia and trophectoderm of the embryo, followed by adhesion amongst these epithelial surfaces. Subsequent invasion of the trophectoderm occurs through the endometrial luminal epithelium, which allows the embryo to embed within the endometrial stroma. Factors secreted at the embryo-maternal interface are critical for supporting the implantation process and for establishing a successful pregnancy.
LEGAL TERMINOLOGY	**DEFINITION**
Human embryo *(Research Involving Human Embryos Act, Australia (2002))*	"… 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."
Human embryo *(Embryo Act, The Netherlands (2002); Medically Assisted Reproduction and the Disposition of Supernumerary Embryos and Gametes Act, Belgium (2003))*	"… a cell or cluster of cells with the potential to develop into a human being."

Human embryo *(Law 14/2007 on Biomedical Research, Spain (2007))*	"… a phase of embryonic development from the moment in which the fertilised oocyte is found in the uterus of a woman until the beginning of organogenesis, and which ends 56 days from the moment of fertilisation, with the exception of the computation of those days in which the development could have been stopped."
Human embryo *(Human Fertilisation and Embryology Act, United Kingdom (1990))*	"(a) … a live human embryo where fertilisation is complete, and (b) references to an embryo include an egg in the process of fertilisation, and, for this purpose, fertilisation is not complete until the appearance of a two cell zygote."
Human embryo *(Human Fertilisation and Embryology Act, United Kingdom (2009))*	"(a) … a live human embryo and does not include a human admixed embryo, and; (b) references to an embryo include an egg that is in the process of fertilisation or is undergoing any other process capable of resulting in an embryo."
Human embryo *(Embryo Protection Act, Germany (2011))*	"… the human oocyte, fertilized and capable of developing, from the time of the fusion of the nuclei, and further, each totipotent cell removed from an embryo that is able to divide and to develop into an individual under the appropriate conditions for that."
Pre-embryo *(Law 14/2007 on Biomedical Research, Spain (2007))*	"… the embryo constituted *in vitro*, derived from the group of cells resulting from the progressive division of the oocyte until 14 days after fertilization."
ETHICAL TERMINOLOGY	**DEFINITION**
Conceptual analysis	The activity of clarifying what concepts mean and imply.
Normative analysis	The activity of producing or assessing the soundness and justifiability of arguments with reference to normative (moral or legal) questions.
Regulatory analysis	The activity of examining rules or systems (e.g. legal norms, professional codes of conduct, etc.) relevant to an activity or process.
The proportionality criterion	The ethical principle according to which the burdens of an activity (e.g. research) must be proportional to the benefits it yields for it to be morally acceptable.
The subsidiarity criterion	The ethical principle according to which the benefits of an activity (e.g. research) must be acquired through the morally least problematic means for it to be morally acceptable.
Active (inherent) potentiality	The view that the potential to develop into a human being is the inherent power possessed by the human embryo to undergo changes to itself.
Passive (contingent) potentiality	The view that the potential to develop into a human being is a contingent possibility that depends upon a series of external events and/or actors.

FERTILIZATION-BASED EMBRYOS: A FOUNDATION FOR UNDERSTANDING EARLY HUMAN DEVELOPMENT

Hertig and Adams (1967) and **Hertig and Rock (1973)** were the first to observe and characterize early human embryos *in vivo*. Since then, our fundamental understanding of early human development has primarily stemmed from *in vitro* fertilization (IVF) in the context of ART. Although limited to the preimplantation period, IVF has certainly provided valuable morphokinetic and metabolic insights into human embryogenesis. However, the signalling interactions and fate diversification mechanisms that accompany these events remain obscure.

To investigate human post-implantation development, efforts have enabled the

extended *in vitro* culture of human embryos beyond the implantation stages. O'Leary and colleagues were the first to examine whole human embryos cultured beyond the blastocyst stage *in vitro* during stem cell derivation, up to 13 days post-fertilization. The reported outgrowths contained the post-ICM intermediate, a structure presumed to closely resemble the human peri-implantation epiblast **(O'Leary et al. 2012; O'Leary et al. 2013)**. Based on mouse systems of post-implantation development **(Hsu 1979; Bedzhov et al. 2014)**, subsequent models allowed extended culture up to 14 days, further attesting to the remarkable ability of the early embryo to self-organize, even in the absence of maternal tissues **(Deglincerti et al. 2016b; Shahbazi et al. 2016)**. Embryo outgrowths showed comparable morphology to early *in vivo* post-implantation human embryos. Specifically, a clear distinction between the epiblast and PE was observed, while putative amniotic and yolk sac cavities were also described **(Deglincerti et al. 2016b; Shahbazi et al. 2016)**. Nevertheless, due to the flattened structure of the outgrowths, these cavities did not expand.

The ability to culture embryos beyond the implantation stages sparked debate regarding the 14-day culture rule for research on human embryos *in vitro* **(Hyun et al. 2016)**. This time point signifies the formation of the PS in human embryos and is also the last stage at which twinning may occur, or at which two embryos can merge (i.e. tetragametic chimerism) **(Table IV)**. Nevertheless, the possibility of extended *in vitro* human embryo culture beyond the PS stage remains unknown. Although extended culture systems are a powerful tool for studying the peri-implantation period, certain limitations still persist **(Rossant 2016)**. The central drawback of the established models is that embryo outgrowths are predominately flattened, which considerably confounds identification of 3D structures formed during normal embryogenesis. Further improvements in culture conditions and the use of refined extracellular matrices may allow greater precision in mimicking physiological conditions. Models involving other species, evolutionarily closer to human, are more amenable to high-resolution technologies, recently providing interesting new data **(du Puy et al. 2011; Kuijk et al. 2012; Sasaki et al. 2016; Kobayashi et al. 2017; Ma et al. 2019; Niu et al. 2019; Taniguchi et al. 2019)**.

In vitro models of embryogenesis

The study of embryogenesis is traditionally based on observing and manipulating human and animal embryos directly. In contrast, the field of synthetic embryology is focused on building ELS from different PSC types *in vitro*. Modulating pluripotency pathways has supported the generation of PSC lines of specific embryonic origin **(Fig. 1, Table IV)**. These have been utilized in two-dimensional (2D) and three-dimensional (3D) settings to mimic various stages of early development **(Table V)**.

Table V: 2D and 3D settings to mimic early embryonic stages.

Developmental stage	Embryo-like structure	Composition	Species	Study
Preimplantation	ETS-blastoids	PSCs and TSCs	Mouse	Rivron et al. (2018)
	Induced blastocyst-like cysts (iBLCs)	PSCs	Mouse	Kime et al. (2019)
	EPS-blastoids	EPS cells	Mouse	Li et al. (2019)
Post-implantation /gastrulation	ETS-embryos	PSCs and TSCs	Mouse	Harrison et al. (2017)
	ETX-embryos	PSCs, TSCs, XEN cells	Mouse	Sozen et al. (2018) Zhang et al. (2019)
	Gastruloids (micropatterned PSC colonies)	PSCs	Human	Warmflash et al. (2014) Etoc et al. (2016) Yoney et al. (2018) Martyn et al. (2018) Tewary et al. (2019)
	Post-implantation amniotic sac embryoid (PASE)	PSCs	Human	Shao et al. (2017) Zheng et al. (2019)
	Gastruloids (3D elongated EBs)	PSCs	Mouse	van den Brink et al. (2014) Beccari et al. (2018) van den Brink et al. (2020)

PSC(s), pluripotent stem cell(s); TSCs, trophoblast stem cells.

Capturing cellular potency *in vitro*

Progress in PSC research started with the observation that teratocarcinoma cells were able to form heterogeneous cell masses (tumours) containing differentiated tissue-like structures following intraperitoneal transplantation in mice **(Pierce and Dixon 1959; Stevens 1959)**. Accordingly, mouse embryonal carcinoma cells were the first PSCs described **(Kleinsmith and Pierce 1964; Martin and Evans 1975)**. These discoveries inspired the derivation of both mouse (mESCs) and human embryonic stem cells (hESCs) from blastocysts **(Evans and Kaufman 1981; Martin 1981; Thomson et al. 1998) (Fig. 1)**. Remarkably, however, hESCs required different culture conditions and showed a distinct epithelial morphology, compared to mESCs. Unlike mESCs that displayed characteristics similar to the preimplantation ICM, hESCs expressed genes of the post-implantation epiblast. Several years later, mouse epiblast stem cells (mEpiSCs) derived from post-implantation embryos, were described to share many similarities with hESCs, including culture conditions, epigenetic state and marker genes **(Brons et al. 2007; Tesar et al. 2007)**. Ultimately, this led to the view that pluripotency exists in at least two states, naive (mESCs) and primed (hESCs and mEpiSCs) **(Table IV)**. In subsequent years, the paradigm of obligatory directional differentiation changed with the discovery of human somatic cell nuclear transfer (SCNT) **(Tachibana et al. 2013)** and particularly, the reprogramming of differentiated somatic cells into induced pluripotent stem cells (iPSCs) **(Takahashi et al. 2007) (Table IV)**. With this technology, many new opportunities for developing patient-specific applications were explored.

The establishment of PSCs (ESCs and iPSCs) made it possible to further characterize

pluripotency and study the molecular mechanisms of differentiation *in vitro*. PSCs have the potential to contribute towards all embryonic derivatives; however, they are limited in their capacity to form extraembryonic tissues. In mouse, the developmental potency of the PE and TE have been captured following the derivation of extraembryonic endoderm stem cells (XEN cells) **(Kunath et al. 2005)** and trophoblast stem cells (TSCs) **(Tanaka et al. 1998) (Fig. 1, Table IV)**. In human, self-renewing TSCs were established from both human blastocysts and villous cytotrophoblast cells **(Okae et al. 2018) (Fig. 1)**. The resulting human TSCs resemble primary trophoblast cells, however further functional analysis will reveal their likeness to *in vivo* counterparts. Interestingly, primary human TE-like spheroids have been generated from hESCs and provide a useful model for evaluating embryo attachment to various endometrial-like cells **(Lee et al. 2015)**. Nevertheless, the extent to which the adhesion of TE-like spheroids truly mimic the complex mechanisms employed by the blastocyst remains to be elucidated **(Weimar et al. 2013)**. Additionally, a recent report described the derivation of extended PSC (EPS) lines in both mouse and human **(Yang et al. 2017)**. EPS were shown to have a totipotent-like developmental potential, maintaining the capacity to contribute towards both embryonic and extraembryonic lineages **(Yang et al. 2017) (Table IV)**. Remarkably, expandable PE-like cells have also been obtained in human, albeit only from hESCs, showing characteristics of extraembryonic endoderm, similar to mouse XEN cells **(Table IV)**. However, as these cells were derived from hESCs *in vitro*, their exclusive extraembryonic character remains difficult to verify functionally **(Linneberg-Agerholm et al. 2019)**.

Modelling mouse preimplantation development: blastocyst-like structures

The establishment of different PSCs *in vitro* has been fundamental for modelling preimplantation development. Nevertheless, current models remain limited to the mouse. In particular, cultured PSCs have been used to generate blastocyst-like structures (also referred to as blastoids or induced blastocyst-like cysts, iBLCs) **(Rivron et al. 2018; Kime et al. 2019; Li et al. 2019) (Table V)**.

Interestingly, blastocyst-like structures were first observed, following the derivation of mouse germ cells *in vitro* **(Hübner et al. 2003)**. Mouse ESCs developed into mature oocyte-like cells that later formed *in vitro* structures resembling mouse preimplantation embryos. More recently, **Rivron et al. (2018)** reported the generation of mouse blastoids by the sequential aggregation of mESCs and mouse TSCs (mTSCs), later referred to as ETS-blastoids **(Li et al. 2019)**. ETS-blastoids were generated in microwell arrays and resemble mouse blastocysts in terms of size, morphology and gene expression. Upon introduction into the uterus of pseudo-pregnant mice, ETS-blastoids induced decidualization, however poorly developed into post-implantation structures both *in vivo* and *in vitro*. As such, these structures are not viable and cannot be used for studying post-implantation development. Their incapacity to develop beyond

the implantation stages may be attributed to a defect in the PE, which, as the authors demonstrated, did not develop properly **(Rivron et al. 2018)**. A recent preliminary study, in which the blastoid culture was optimized to form a PE-like compartment more efficiently, indeed showed enhanced survival and morphogenesis of blastoid-derived post-implantation ELS *in vitro* **(Vrij et al. 2019)**. Possibly a critical factor resulting from the absent PE is the related absence of the Reichert's membrane and parietal yolk sac.

Kime et al. (2019) further generated blastocyst-like structures starting from mPSCs only. The formation of iBLCs relied on the establishment of progenitor cells that express the totipotency-related, cleavage-stage marker MERVL **(Macfarlan et al. 2012)**. Like ETS-blastoids, the generated iBLCs morphologically resembled mouse blastocysts, induced decidualization in pseudo-pregnant mice and similarly did not develop further **(Kime et al. 2019)**. A further study revealed that it is also possible to generate blastocyst-like structures from mouse EPS (mEPSCs). EPS-blastoids, consist of all three cell lineages (epiblast, TE and PE cells) **(Li et al. 2019)**. Like ETS-blastoids and iBLCs, EPS-blastoids also resembled mouse blastocysts in terms of size and morphology, showed potential to induce decidualization in pseudo-pregnant mice but underwent resorption a few days after transfer. Interestingly, the authors were also able to generate clonal EPS-blastoids from a single EPS cell, albeit at a very low efficiency (2.7%). EPS-blastoids were also generated from iPSCs, serving as a proof of principle that blastocyst-like structures can be generated from somatic cells **(Li et al. 2019)**.

Preimplantation ELS may serve as a valuable model for elucidating the expansion of the mouse blastocoel cavity and the interaction between mPSCs and mTSCs. As aforementioned, blastocyst-like structures have not been developed from human cells thus far; however, research in coming years is likely to follow a similar path as in mouse.

Modelling mouse post-implantation development: ETS/X embryos

Aspects of mouse post-implantation development have also been captured *in vitro*. Aggregates of mESCs and mTSCs cultured in a 3D extracellular matrix-like scaffold have been shown to self-assemble and acquire a cylindrical architecture, similar to that of the early post-implantation embryo **(Harrison et al. 2017)**. These structures were coined ETS-ELS (ESC and TSC ELS). ETS-ELS showed signs of asymmetric induction of mesoderm and specification of cells resembling primordial germ cells in their gene expression patterns. However, due to the absence of extraembryonic endoderm, further (proximal–distal) patterning could not be achieved. In a further study, the combination of mESCs, mTSCs and mouse XEN cells led to ELS that recapitulated gastrulation-like events, including anterior–posterior and proximal–distal patterning, epithelial to mesenchymal transition (EMT) **(Table IV, Table V)** and the formation of definitive endoderm **(Sozen et al. 2018)**. These so-called ETX-ELS (ESC, TSC and XEN cell ELS) aggregated spontaneously under shaking-conditions **(Zhang et al. 2019)**, indicating a certain level of self-organizing properties of mouse development. Following transfer

into pseudopregnanct mice, ETS or ETX-ELS-induced decidualization, but degraded after 3 days **(Zhang et al. 2019)**. Ultimately, this is to be expected as mouse implantation starts at the blastocyst stage, resulting from the interaction of mural TE cells with the endometrium. These cells are not maintained in the ETS and ETX-ELS. Moreover, this highlights the importance and robust nature of the decidualization reaction.

To date, the generation of human ETS or ETX-ELS has not been described. Due to the geometrical differences between mouse and human peri-implantation embryos, it would be preferable to develop a model with hESCs and XEN cells inside a layer of TSCs, instead of hESCs and TSCs inside a layer of XEN cells, used to model the mouse.

Modelling human post-implantation development: micropatterns

Manipulating the microenvironment of differentiating PSCs by confining them to micropatterns or within 3D networks has been shown to simulate early morphogenesis *in vitro* in a more reproducible manner. It has been established that cell fate can depend on the size of stem cell colonies **(Lee et al. 2009)** and research efforts have been directed towards culturing PSCs in geometrically confined colonies **(Peerani et al. 2009)**. For this, cells are deposited on circular-shaped micropatterns to which they can attach **(Deglincerti et al. 2016b)**. Generating micropatterned colonies ensures both reproducibility, as well as a quantitative platform for (imaging) analysis using well-established algorithms to determine lineage restriction and cell movement in real time. Using this method, **Warmflash et al. (2014)** demonstrated that micropatterned colonies of hESCs (also referred to as human gastruloids) cultured in the presence of BMP4 acquired fates of the three germ layers and TE, which were radially organized. Moreover, cell fate depended on the distance from the colony edge, coinciding with the localization of BMP4 receptors **(Warmflash et al. 2014; Etoc et al. 2016; Yoney et al. 2018)**. In a further study, micropatterns were used to construct a rudimentary fate map of the human PS. However, in the absence of human data, comparisons with the mouse embryo were used to define markers for human cell types **(Martyn et al. 2019)**.

Overall, micropatterns have proven advantageous for studying size regulation and signalling requirements of hPSCs during gastrulation. However, the morphology of these 2D cultures does not resemble that of the human embryo, in which the three germ layers are positioned on top of each other, with a surrounding TE. Moreover, bilateral symmetry was not established *in vitro*. Nevertheless, these models have certainly set the stage for future work using geometrical control to study differentiation dynamics in a highly quantitative manner. Work on combining PSCs with synthetic or tissue-derived scaffolds in combination with sophisticated bioengineering methods may ensure greater feasibility for modelling the development of complex tissues.

Modelling human post-implantation towards gastrulation: 3D ELS

Research encompassing 3D models of the human embryo has also achieved remarkable

progress in recent years. In human, advancements have been made in generating structures mimicking the amniotic cavity. **Shao et al. (2017)** modelled amniogenesis by using a 3D culture system to induce hESCs and hiPSCs to self-organize into structures that closely resembled the human amnion and ectoderm, termed 'post-implantation amniotic sac embryoid' or PASE **(Shao et al. 2017)**. The defined number of PSCs and the culture medium employed led to an asymmetric structure containing epiblast-like cells and cells resembling the amniotic ectoderm. This structure remained stable even as the cells divided. This model represented the first platform to study human early development *in vitro*. In a further study, **Simunovic et al. (2019)** used micropatterned systems in a 3D setting to generate an *in vitro* 3D model of the human pre-gastrulation epiblast. The epiblast-like structure was shown to spontaneously break symmetry under a uniform dose of BMP4, further expressing early markers of the PS and EMT, suggesting initiation of gastrulation **(Simunovic et al. 2019)**.

Building on the PASE model, **Zheng et al. (2019)** further improved efficiency and reproducibility by using microfluidics to achieve a controllable system for recapitulating events involved in epiblast and amniotic ectoderm development. The hPSCs adopted a 3D organization that mimicked the formation of the amniotic sac. Remarkably, by modifying the culture conditions, the authors were able to induce axis formation and gastrulation-like events, observing cell populations that resembled PS-like cells and endoderm-like cells, as well as human primordial germ cell-like cells. This model is one of the first systems to capture the complexity of spatial relationships and cellular interactions during early post-implantation development and gastrulation in human **(Clark 2019)**.

Modelling gastrulation towards early organogenesis in mouse: embryo bodies and gastruloids

To date, gastrulation has been mapped in several model organisms, such as mouse, allowing the fate and location of specific cell types to be characterized **(Vogt 1929; Hatada & Stern 1994; Tam & Behringer, 1997; Alev et al. 2010)**. However, no such descriptions have been made in human, primarily due to ethical constraints on embryo culture (14-day rule). Yet a map of the gastrulating human embryo would be of immense value for providing more comprehensive insights into human development and allowing for species-specific differences to be evaluated. Moreover, a greater understanding of cell fate specification would inherently contribute to PSC differentiation efforts.

In a developmental context, embryoid bodies (EBs) have long served as a standard tool to assess PSC pluripotency, as aggregates of PSCs are able to differentiate spontaneously into the three germ layers **(Table IV) (Desbaillets et al. 2000; Itskovitz-Eldor et al. 2000)**. However, spontaneous differentiation of PSCs into EBs usually results in heterogeneous cell populations and terminally differentiated cells are rarely obtained, primarily due to the lack of control of each differentiation step. Nevertheless,

in specific culture conditions, mouse EBs have been used to generate ELS that mimic symmetry breaking events observed during early post-implantation development **(ten Berge et al. 2008; Fuchs et al. 2012)**. Modified culture protocols have enabled key aspects of post-implantation mouse development to be captured *in vitro* **(Marikawa et al. 2009; van den Brink et al. 2014; Turner et al. 2017; Beccari et al. 2018)**. Analysis of these ELS using immunofluorescence and at the single-cell level revealed that they formed the three germ layers with reference to the three body axes **(Beccari et al. 2018; van den Brink et al. 2020)**. As these processes are the consequence of gastrulation, the structures were termed gastruloids. **Beccari et al. (2018)** revealed that mouse gastruloids mimic the spatial and temporal patterns of Hox gene expression that determines the anteroposterior organization of the embryo. Mouse gastruloids can be used to study gastrulation, body axis establishment and the early phases of organogenesis in an animal-free and high-throughput manner *in vitro*. However, they do not generate anterior neural (brain) cells or any extra-embryonic tissues, and are not able to implant in utero, ultimately lacking full organismal potential. Although specific culture conditions can induce differentiation of gastrula organizer cell in hESC-derived EBs **(Sharon et al. 2011)**, no human version of these 3D-gastruloids has been reported thus far.

Limitations and challenges of ELS

PSC-based ELS represent an important addition to the human stem cell toolbox **(Clark 2019)**. However, these structures cannot fully develop and progress into viable embryos. ELS primarily lack critical cell types and cellular organization, leading to defective development. Furthermore, current ELS remain largely limited to the mouse, and the extent to which they recapitulate the transcriptomic and epigenetic signature of both *in vitro* and *in vivo* fertilized embryos is yet to be elucidated. Moreover, the crosstalk at the embryo-maternal interface that actively assists in the final stages of pre- and early post-implantation development **(Gellersen & Brosens 2014)** is ultimately abnormal between ELS and the maternal uterus. As seen in mouse ELS studies, this leads to developmental arrest a few days after implantation. The complex yet crucial signals resulting from the interactions between the embryo and the surrounding maternal environment are yet to be uncovered and reliably captured *in vitro*. To date, the blastocyst is the only stage compatible with implantation, while proper interactions with the maternal endometrium are an essential prerequisite for further development **(Evans et al. 2012; Koot et al. 2012)**.

Concurrent to scientific innovation, considering the extent to which the use of ELS raises ethical challenges related to human embryo research remains paramount. This will be crucial for harnessing the potential of ELS as a valuable research tool, whilst also remaining within a robust moral and legal framework of professionally acceptable practices. Ultimately, the extent to which ELS actually mimic fertilization-based embryos

will govern the nature of the ethical and regulatory issues that stem from their creation and use **(Pera 2017)**.

RELEVANT ETHICAL AND POLICY ASPECTS OF THE HUMAN EMBRYO RESEARCH DEBATE

The increased opportunity of studying early human life in a dish ever since the advancement of IVF did not only hold great scientific and therapeutic promise, but it also raised a series of challenges about instrumental and destructive human embryo research **(Mulkay 1994)**. The public was "divided between pride in the technological achievement, pleasure at the new-found means to relieve, at least for some, the unhappiness of infertility, and unease at the apparently uncontrolled advance of science, bringing with it new possibilities for manipulating the early stages of human development" **(Warnock 1984)**. Robust moral and legal directives capable of enforcing professionally acceptable practices were requisite in addressing these concerns.

Conceptual debate: the concept of 'the embryo'

The stipulation of necessary and/or sufficient conditions for an entity to qualify as a human embryo was particularly crucial to this end: before knowing how, one must know what, to regulate. An interaction between the natural sciences and the humanities on how to (re)define the human embryo and its constituent criteria was therefore essential.

The rise and fall of the 'pre-embryo'

In the past, little thought had been given to an exact definition of the 'embryo'. It was obvious that the mammalian 'embryo' referred to the developing entity resulting from the *in vivo* fertilization of an oocyte by a spermatozoon. Embryologists also commonly used the term interchangeably with 'ovum' or 'conceptus'; terms derivative from studies with invertebrates and other lower animals meant to designate "the totality of cells derived from the fertilized egg" **(McLaren 1986)**.

As knowledge of embryology progressed, it became clear that, in early mammalian embryogenesis, the cells derived from the fertilized oocyte commit to distinct fates. Whereas a vast majority of these cells will develop into extraembryonic structures, only a small part of the ICM will actually transform into the so-called 'embryo-proper' or 'definitive embryo' **(McLaren 1986)**. Prior to cellular differentiation, referring to the developing entities as 'embryos' thus seemed to be "no more (maybe less) appropriate than to refer to them as placentae" **(McLaren 1987)**. Several alternative terms have since been put forth in an effort to represent morally relevant differences in (extra) embryonic fates, with the 'pre-embryo' becoming by far the most popular one in

scientific and ethical literature.

For critics, however, the conceptual distinction between embryos and pre-embryos conveyed the impression that they were also normatively different, i.e. that the latter were morally inferior to the former. The critique that the term defined moral problems away, ultimately led to its gradual abandonment before the mid-1980s. With notable exceptions, such as Spain **(Table IV)**, most other jurisdictions have since returned to 'embryo' as the designated term to denote the beginnings of early (human) life. Nevertheless, it remained necessary to specify the exact criteria the term conveys. This was clearly no easy task, as the conditions for embryo qualifications continue to vary considerably across jurisdictions **(European Group on Ethics in Science and New Technologies 1999)**.

Conditions for embryo conceptualizations: fertilization and potential

In the past, fertilization was, and in some jurisdictions still is, presumed to be a necessary and sufficient condition for entities to qualify as embryos. The Human Fertilization and Embryology (HFE) Act 1990, for instance, referred to the embryo as "a live human embryo where fertilization is complete" **(Human Fertilization and Embryology Act 1990) (Table IV)**. Another example is the presently enforced Spanish Law 14/2007, which still defines the embryo as the "fertilized oocyte" **(Law 14/2007 of July 3th on Biomedical Research, 2011) (Table IV)**. Interestingly, while both examples refer to the same phenomenon as a point of reference for embryo conceptualizations, they are discrepant with regard to the intended phase of development. While the HFE Act demarcates the two-cell stage as the completion of fertilization, the Spanish Act fails to specify an exact phase, alluding to fertilization instead as a single, isolated 'moment' in embryogenesis. This variance can have important implications for regulation. Rather than being defined as a moment, fertilization is more appropriately understood as a process; i.e. a series of consecutive events in the 20–22 h following the meeting of an oocyte and a sperm, which include, among others, syngamy and zygotic genome activation **(National Health and Medical Research Council 2006)**. What, then, is the fundamental event in fertilization from which to begin counting? Fertilization-based embryo conceptualizations, if unspecified, prove too ambiguous for suitable, clear-cut directives.

Since Dolly's birth, the cloned sheep, also implied the possibility of producing offspring through SCNT in humans **(Table IV)**, it was widely felt that fertilization could no longer be regarded as a necessary condition for embryo qualifications **(Health Council of the Netherlands 2005; Piotrowska 2019)**. Countries that have maintained it while also ratifying the Oviedo Convention (which forbids creating human embryos for research **(Council of Europe 1997)**), make themselves vulnerable to charges of duplicity, allowing them to appear supportive of embryo protection while giving free reign to SCNT-research **(Beriain 2014; Dondorp & de Wert, 2017)**.

In most jurisdictions, post-Dolly definitions of the human embryo have thus either come to refer to fertilization as a sufficient condition amongst others or substituted it altogether. An example of the former is Australian legislation, which widened the definition to include entities deriving from "any other process that initiates organized development" **(National Health and Medical Research Council 2006) (Table IV)**. Whereas the Australian definition still contains an implicit reference to fertilization, any such reference is abandoned in Dutch and Belgian legislation, which define the human embryo as "a cell or cluster of cells with the potential to develop into a human being" **(Embryo Act 2002; Medically Assisted Reproduction and the Disposition of Supernumerary Embryos and Gametes Act 2003) (Table IV)**. Moreover, whereas the Australian definition looks at commencing development (limited to the first eight weeks of development since the first mitotic division), the Dutch and Belgian definition focuses on its completion.

Dutch and Belgian regulators do not further specify the exact meaning of 'the potential to develop into a human being'. Commentators have pointed out that, when this is understood as stretching unto birth, it legally implies that the concept of non-viable embryos is a *contradictio in terminis* **(de Wert & Mummery 2003)**. Similar disputes have emerged in two rulings of the Court of Justice of the European Union **(Judgment of 18 October 2011; Judgment of 18 December 2014)**. In the *Brüstle vs Greenpeace* case, the CJEU ruled that organisms "capable of commencing the process of development of a human being" **(Judgment of 18 October 2011)** were to be regarded as human embryos for the purpose of the relevant EU-Directive. This would apply to fertilized oocytes, oocytes activated through parthenogenesis, and oocytes into which the nucleus of a mature human cell was transplanted (SCNT). When the relevant criterion was challenged with regard to parthenotes in the International Stem Cell Corporation case, the CJEU requalified its earlier ruling, stipulating that "in order to be classified as a 'human embryo', a non-fertilized human oocyte must necessarily have the inherent capacity of developing into a human being" **(Judgment of 18 December 2014)**.

At stake here, is that each criterion (fertilization, commencing development or the potential to develop into a human being) differentially determines the scope for the applicability of embryo protective regulations and related normative debates. For instance, in the Dutch debate, it has been argued that the legal definition may be both too narrow and too wide **(Winter et al. 2012)**. Too narrow because it preempts the question of whether non-viable human embryos might also deserve some level of protection. Too wide because gametes and, with present technologies, even somatic cells, may also be said to have the potential to develop into a human being. Whether this implication can be avoided by the added qualification 'inherent' (as in the last quoted CJEU ruling) is a matter for debate, to which we return below.

Normative debate: the moral standing of early human life

In the words of Mary A. Warren, to have moral status is...

> "... to be an entity towards which moral agents have, or can have moral obligations. If an entity has moral status, then we may not treat it in just any way we please; we are morally obliged to give weight in our deliberations to its needs, interests or wellbeing. Furthermore, we are morally obliged to do this not merely because protecting it may benefit ourselves or other persons, but because its needs have moral importance in their own right" **(Warren 1997)**.

Sentient animals have moral status in this sense because, as entities capable of feeling pain and experiencing discomfort, they have interests that can be thwarted. Although the same goes for human beings, it is generally only with reference to them that the term 'full moral status' is used.

The idea is that there is more to humans than the features they share with (other) sentient animals. Of course, there are different interpretations of what this 'more' entails, ranging from the belief that human beings are created in God's image to the claim that humans are persons, i.e. that they have certain properties (such as self-consciousness, rationality and the capacity for intentional action) that command respect because they are essential for moral agency **(DeGrazia 2012)**. Thus, whereas the instrumental use of animals may, under conditions of proportionality and subsidiarity **(Table IV)**, be reconcilable with acknowledging their moral standing, it is generally argued that, given their full moral status, human beings should never be treated as mere means **(Kant 1998)**.

Now, what about the moral status of human embryos? Do they qualify as entities deserving of protection in their own right? If so, what level of protection do they deserve and what does that imply for their research use? This discourse has traditionally focused on the status of preimplantation embryos due to the former inability of culturing human embryos *in vitro* for longer than a few days. Here, three broad views can be discerned along a spectrum **(Robertson 1986)**. At one end is the view that, at preimplantation stages of development, the embryo is morally equivalent to a mere cluster of cells. This view "allows the embryo to be treated like any other human tissue used in research, subject only to rules to protect the interests of those who have dispositional control of the embryo" **(Robertson 1986)**. At the other end are those who view preimplantation embryos as deserving the same moral reverence due to any human being **(Ford 1988)**. The rationale for this position, which is generally grounded in Christian beliefs, is that what makes human beings morally special also applies to human embryos from the earliest stages of development. Since full moral standing involves full protection, the resultant policy is to ban embryo research altogether, except for research aimed at benefiting the embryo itself. Between these extremes, the dominant view regards

"the embryo as deserving of respect greater than that accorded to tissue, but not as much as that accorded to persons" **(Robertson 1986)**. This view is often understood as gradualist, according human embryos an initially low but increasing moral status with certain developmental milestones **(National Institutes of Health 1994; Hermeren 1996)**. As such, it is tolerant of embryo research, albeit under conditions of proportionality and subsidiarity.

The question is, of course, on what basis the third view accords this relative moral status to preimplantation embryos. Bearing in mind that the properties generally regarded as morally significant in the partly overlapping abortion debate, e.g. heartbeat, sentience or incipient brain activity **(Tauer, 1997)**, have not yet emerged at this stage of development, the most promising feature appears to be their potential to grow into a human being.

The argument from potential

Ascriptions of moral status based on the embryo's potential to develop into a human being may depart from very different understandings of potentiality **(Buckle 1990)**. Those reasoning along the lines of classical Aristotelian teleology will understand this notion as 'active' or 'inherent' potentiality, i.e. as the organism's intrinsic power to become what it is destined to be. Assuming that the embryo is destined to become a fully developed human being, and given that (as persons) human beings are morally worthy ends, this active potential is what entitles it to moral standing. However, depending on whether the emphasis lies on the continuity or the difference aspect of the potential to self-realization, this may still lead to very different conclusions. Those emphasizing continuity, i.e. the view that the embryo is already the human person that it will become, will maintain that the fertilized oocyte has the same (full) moral status as any of us and may not be used as research material. Those emphasizing the still large difference between what the embryo is and what it will become, will accord it a much more limited moral status, allowing its instrumental use under certain conditions.

The embryo's potential to develop into a human being may alternatively be understood as 'passive' or 'contingent': as merely one of many possible outcomes, all of which ultimately rest on external events or actors. Those reasoning from this perspective point out that a fertilized oocyte has actually a greater chance of perishing than of it ever becoming a human being, thus making it difficult to take intrinsic-potentiality arguments seriously. Furthermore, if the fertilized oocyte has moral status by dint of its intrinsic potential to become a human being, then should not the oocyte and sperm, which combined have the same potential, be conferred equivalent moral standing? **(Kuhse and Singer 1982)**.

However, this *reductio ad absurdum* misunderstands the meaning of active potentiality. Given that this notion refers to "the power possessed by an entity to undergo changes … to itself" **(Buckle 1990)**, it requires the preservation of individual

identity throughout its actualization. Entities can therefore only possess the morally relevant potential from the moment they can be identified as numerically identical to the human being they will become. This impedes the ascription of active potentiality not only to gametes, but also to fertilized oocytes and preimplantation embryos since, at these 'pre-embryo' stages, the cells from which the embryo-proper develops are still indistinguishable from those that will take on extraembryonic functions. As a consequence, it is only from the development of the embryo-proper onwards, which is also the stage from which natural twinning can no longer occur **(Table IV)** that embryos can be said to possess the potential required for moral standing. While this reasoning may save active potentiality from the reductio-charge, it comes at the price of limiting its applicability to post-implantation embryos. At earlier stages, only passive potential can then be ascribed.

If prior to the emergence of the embryo-proper the argument from potential can indeed only refer to passive potentiality, this need not mean that there are no grounds for ascribing moral status to preimplantation embryos. Doing so, however, must then be a matter of granting early human life a certain degree of symbolic value or moral standing by association, rather than acknowledging any intrinsic moral value.

Conditions for acceptable embryo research

The so-called 14-day rule limits human embryo research to 14 days of development or equivalent developmental stages, such as the beginning of gastrulation or the formation of the PS **(Warnock 1984; Appleby & Bredenoord 2018)**.

The specific stipulation of this limit ensued from moral deliberation on the biological qualities of early human embryos. Of special relevance were considerations regarding the beginnings of neurulation **(Table IV)**, with which sentience is associated, and ontological individuation. Whereas neurulation occurs between 17 and 26 days after fertilization, natural twinning can still occur until 14–15 days after fertilization **(Table IV) (Warnock 1984)**. Hence, in an effort to err on the side of caution, legislators limited embryo research to stages amply preceding the development of morally concerning features.

The precise reasons for keeping clear of these specific features were never spelled out. As ontological individuation establishes numerical identity between the embryo and the human individual it may grow into, reaching the stage where twinning is no longer possible seems relevant only for those subscribing to the idea of active potentiality. In fact, only for those linking this idea with the ascription of full moral status, would it seem obvious that ontological individuation poses an absolute limit to the moral acceptability of human embryo research. Equally underdetermined are the reasons for steering free from other features of concern. Sentience as such, for instance, is not a sufficient reason for a strict limit, given that research with sentient animals is accepted, albeit conditionally. Moreover, to the extent that features such as

the beginnings of neurulation can be understood as relevant milestones in a gradualist account of moral status (whether intrinsic or symbolic), it is also not obvious why this would require categorically cutting-off research at day 14. The 14-day rule was thus not adopted because there were more convincing reasons for drawing the line here rather than at some later stage, but because it provided a pragmatic means to allay public anxiety while delineating a clear-cut and enforceable boundary **(Warnock 1984, 2007; Cavaliere 2017; Warnock 2017)**.

Despite its widespread and longstanding acceptance, scholars are now appealing for a reevaluation of the rule. At the time of its formulation, the possibility of culturing human embryos *in vitro* for longer than 14 days seemed too far-remote to be taken into consideration. At present, however, it is becoming a feasible reality **(Hyun et al. 2016)**, with scientists having managed to maintain human embryos alive for an unprecedented period of 13 days *in vitro*, after which they had to cut off their experiments **(Deglincerti et al. 2016a)**.

For embryo research to be permissible within this or any alternative timeframe, it must meet the additional conditions of proportionality and subsidiarity **(Table IV)**. The proportionality condition holds that embryo research must serve a morally important goal **(Pennings & van Steirteghem 2004)**. For instance, while the instrumental use of human embryos for the treatment of major diseases is acceptable, their use for the safety testing of cosmetics is not. Subsidiarity requires that no morally less problematic alternatives for reaching the same goal are available **(Pennings & van Steirteghem 2004)**. For instance, human embryos should not be used for research that can be done with non-embryonic cells or tissues, nor should they be created as research materials for studies that can be conducted with supernumerary IVF embryos. While it has also often been taken for granted that this principle favours the use of animals over human embryos, this is less obvious if human embryos have at best a relatively low moral status **(Jans et al. 2018)**.

DISCUSSION

The importance of the scientific developments summarized in this review lies in the double expectation that human ELS may provide accurate means to study embryonic processes, while being sufficiently different from fertilization-based embryos to keep free from restrictions limiting their research use **(Fig. 2)**. There is an obvious tension between these two perspectives that needs further exploration: to what extent can these models be applied, or improved upon, without raising (part of) the concerns behind the barriers that the modelling hoped to circumvent? Despite recent efforts towards the development of tangible guidelines **(Hyun et al. 2020; ISSCR Statement on Ethical Standards for Stem Cell-based Embryo Models 2020)**, the ethical and

regulatory ramifications of this emerging field remain to be thoroughly defined. To this end, the present section juxtaposes the conceptual distinctions and normative positions previously discussed in the context of traditional human embryo research to (specific types of) ELS. Each subsection will focus on elucidating the ensuing conceptual, normative and regulatory implications for ELS (research), respectively.

Are ELS embryos?

As previously shown, there is no universally accepted biological, legal or ethical definition of the human embryo. This means there are different possible answers as to whether (certain) ELS qualify as human embryos. In jurisdictions where fertilization is a necessary condition for such qualification, ELS are clearly not embryos as none of these constructs arise from the fusion of gametes.

If, as in alternative definitions, it suffices that the relevant entity has the potential for embryo-like development, it becomes a matter for debate whether certain ELS qualify as such **(Piotrowska 2019)**. In Australia, for instance, PASE, gastruloids and ETS/X constructs might, whereas blastoids might not **(Table IV)**. If the definition requires the potential to develop into a human being, as defined by Dutch and Belgian legislation, it becomes unclear how to categorize ELS **(Hyun et al. 2020)**. The fact that present-day ELS develop only up to a certain point implies that they cannot (yet) qualify as embryos under this definition. Moreover, defining the embryo in these terms presents an epistemological challenge in that we cannot know whether the definition applies to ELS without conducting experiments that would themselves raise ethical concerns. Would it suffice if animal experimentation were to suggest that improved ELS could lead to offspring in at least other mammals? Similar post-Dolly reasoning seemed to be the basis for regarding SCNT-products as embryos under Dutch and Belgian legislation **(Health Council of the Netherlands 2005)**.

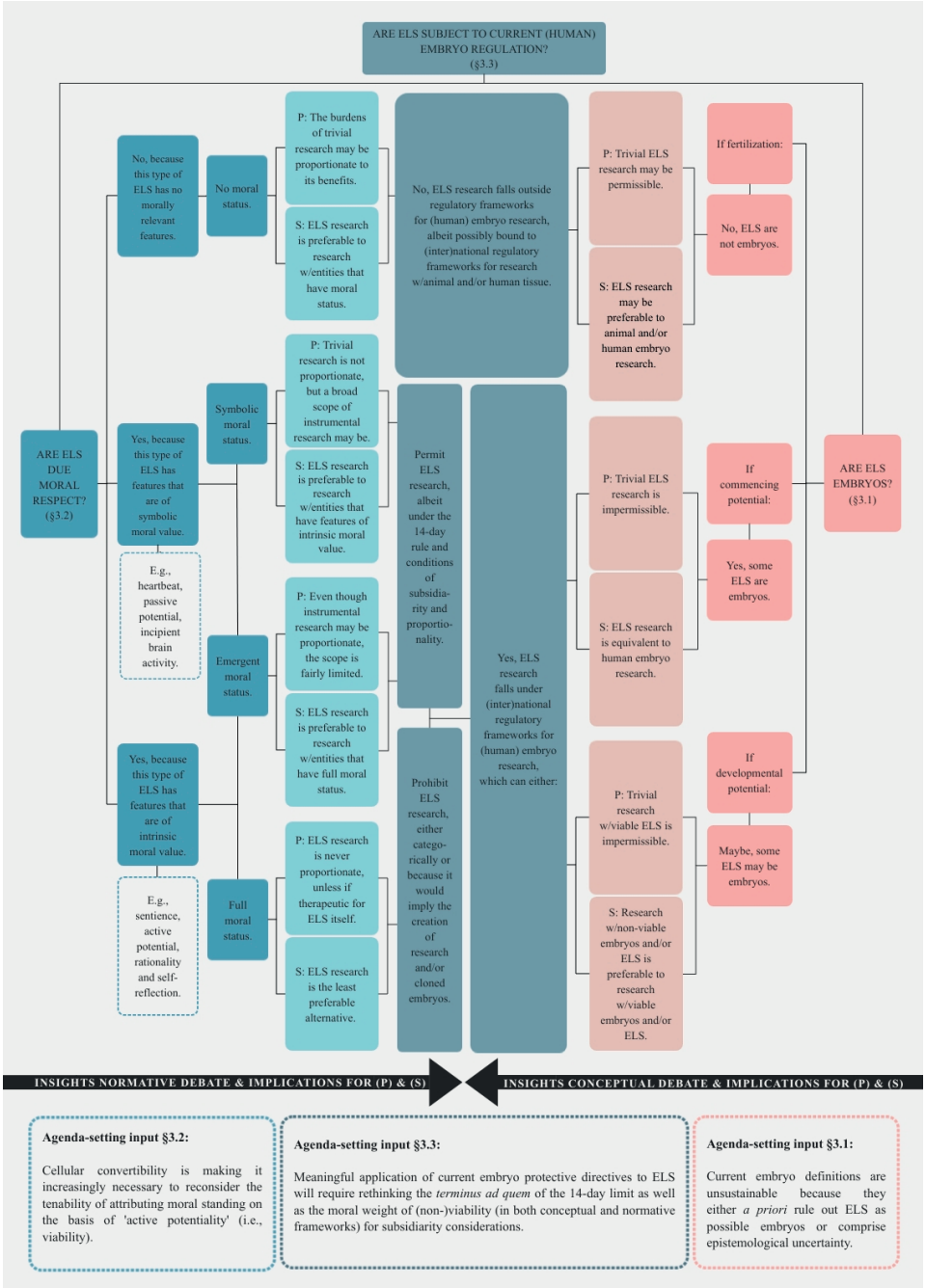

Figure 2: Overview of ethically relevant aspects in the traditional human embryo debate and respective implications for ELS research. Prominent conceptual and normative conditions for the qualification, moral standing and regulation of research with early human life in the traditional human embryo research debate are summarized and extended to (research with) ELS. Subsequent implications for the principles of proportionality (P) and subsidiarity (S) are shown.

Then again, the potential to develop into a human being may be 'switched off', for instance, by knocking out the genes necessary for development beyond a certain stage. As in the earlier SCNT-debate, where it was proposed to use modified stem cells so as to circumvent US public funding rules **(Grompe 2005)**, the ability to switch certain features 'on' and 'off' might be seen as providing a way to ensure that, at least in terms of such-like definitions, ELS can never qualify as embryos **(Pera et al. 2015)**.

Importantly, the question whether ELS qualify as embryos is not just relevant for scientists wanting to determine the ethical and regulatory boundaries of their work, but also the other way around; our analysis shows there is an urgent need for policymakers to reconsider the reasoning behind present definitions in light of current developments. Whereas fertilization-based definitions are clearly too narrow, as they preempt the question whether human beings could develop from processes other than the fusion of gametes, potentiality-based definitions may be too wide, depending on how the notion of 'potential' is understood.

While legislators may have intended 'the potential to develop into a human being' to mean active potentiality, it has been argued that this concept is no longer tenable in view of recent technological advancements **(Stier & Schoene-Seifert 2013)**. In particular, these advancements do not only show how very different types of human cells may be converted into 'baby-precursors', but they also emphasize the extent to which, even in standard human reproduction, embryo development is dependent upon "innumerable external biochemical triggers" **(Stier & Schoene-Seifert 2013)**. In this sense, there appears to be no difference between the potential of a skin cell, a pluripotent stem cell, or a zygote: with the right kind of external triggers, each can be made to develop into a human being. Although this remains a contested position **(Cunningham 2013; Hyun 2013)**, the very debate suggests that, if unspecified, the notion of developmental potential does not provide a solid basis for distinguishing between embryos and non-embryos. Moreover, definitions in terms of this notion also appear to imply that, for the purposes of regulation, there are no such things as non-viable embryos.

Definitions in terms of commencing potential avoid these challenges while remaining conscientious of what scientific practice regards as embryos. On the other hand, the fact that this definition would include ELS that are structurally incapable of developing into a human being, will be regarded by many as casting the definitional net too wide. Of course, the question of whether ELS are to be regarded as embryos should be distinguished from the further question of whether and to what extent they deserve protection. If non-viability does not inhibit ELS from qualifying as embryos, it may still be a good enough reason for according them a lower moral status, thereby allowing more room for their use in research.

Are ELS due moral respect?

First, to say that (certain) human ELS are not embryos does not imply that they cannot

have moral status or that their development cannot raise moral concerns. For instance, should it become possible to develop ELS capable of feeling pain, then, this ability alone grants them a moral standing akin to that of sentient animals. Moreover, regardless of whether or not ELS qualify as human embryos, they may still develop features that many would consider morally concerning, such as incipient brain activity or an emerging human form. Similar issues are found in the context of research with cerebral organoids **(Farahany et al. 2018; Lavazza & Massimini, 2018; Hostiuc et al. 2019)**.

Second, if (certain) ELS are embryos, then, depending on the definition underlying this qualification, they either do or do not have the potential to develop into a human being. Short of becoming sentient, ELS lacking this potential cannot be conferred more than symbolic worth. Conversely and hypothetically speaking, if ELS were to have this potential, the question becomes whether it is understood as passive or active. Passive potentiality would imply that ELS could at best have moral value by association: they owe their moral status to the symbolism associated with what they represent, i.e., the beginnings of early human life. This value may then increase with the achievement of developmental milestones. Active potentiality would imply that ELS have intrinsic value and, therefore, independent moral status. Here, views diverge. For some, ELS with active potential would be due full moral standing, regardless of their developmental stage. For the majority, however, while active potential would confer ELS independent moral status, this status would be initially low and gradually increase with further development.

Moreover, for those who accept the view that active potentiality requires ontological individuation, i.e. that the relevant entity must be the same organism (in terms of numerical identity) as the human being it will develop into, it follows that only ELS capable of modelling post-implantation development (e.g. the 'embryo-proper') could have this potential and respective (intrinsic) moral status. In this view, both ELS limited to modelling stages preceding individuation (such as blastoids), and fertilization (or SCNT)-based embryos at corresponding stages, would thus have symbolic worth at most.

ELS as a policy challenge

If (certain) ELS do not qualify as embryos, it follows that their research use will not be subject to regulations any more than is the case for research with (human) cells and tissues generally. Aside from the fact that this may enable the study of developmental stages for which, given the widespread acceptance of the 14-day rule, human embryos cannot be used, it may also imply that research with ELS ought to be prioritized over research with animals or human embryos from a subsidiarity perspective. Nevertheless, given the possibility of ELS raising moral concerns equivalent to those raised by animal (e.g. sentience) or post-implantation human embryo (e.g. heartbeat) research, there may be a regulatory lacuna here.

If (certain) ELS are embryos, the question arises how current embryo research regulations apply to them. Research with ELS that qualify as such will not only be prohibited in jurisdictions that forbid embryo research altogether (e.g. Germany **(Embryo Protection Act 1990)**) but also in jurisdictions where the creation of embryos for research purposes is not allowed (e.g. all countries that ratified the Oviedo Convention). Some may view this as a further occasion for questioning the 'discarded-created' distinction behind this ban **(Devolder 2005)**, or point to the fact that the 'feminist' argument against creating embryos for research (referring to the burdens and risks of oocyte donation **(George 2007)**) does not apply to the creation of ELS.

A further issue is how ELS research relates to cloning. Depending on whether hESCs or iPSCs cells are used, ELS research may lead to entities that are a genetic copy of either the embryo from which hESCs were derived (i.e. 'embryo cloning') or the individual whose cells were used to create iPSCs (i.e. 'adult cloning'). It is important to keep this in mind because, even though most countries only forbid human reproductive cloning, some also prohibit human cloning for research purposes **(Isasi & Knoppers 2006; Paolo Busardò et al. 2014)**, thereby impeding both SCNT and ELS research.

Another prominent and currently widely debated issue is the elusiveness of applying the 14-day rule to research with entities whose development starts at what for fertilization (or SCNT)-based embryos would be different post-fertilization stages **(Hyun et al. 2020)**. A meaningful application of the rule to ELS would thus require its *terminus ad quem* to refer to morally relevant features rather than the mere duration of development **(Aach et al. 2017)**. In this respect, the explicit reference to the appearance of the PS as an alternative ground for limiting embryo research whenever this would come before reaching the time limit, seems to give the British HFE Act an advantage vis-à-vis articulations only referring to the first 14 days, as the Dutch and Belgian Embryos Acts. In any case, pragmatic arguments for maintaining the 14-day limit must give way to the inevitability of reconsidering the material grounds for regarding the appearance of the PS, or any other subsequent developmental feature, as a point after which human embryo research would be morally unacceptable. The fact that PS formation is a core developmental feature of certain ELS underscores the urgency of this debate. Note that this is not just a matter of the traditional framework falling short of new developments, but also of those developments (further) revealing the indeterminateness of the reasoning behind the 14-day limit as a cornerstone of the traditional framework.

The same is true regarding the requirement of subsidiarity: ELS showcase how current frameworks lack specific regulations for non-viable embryos. Again, depending on the exact definition, non-viable embryos either fall outside the scope of embryo research regulations, or are subject to the same regulations as viable embryos, where neither of them is given priority if both can be used as research material. There may, however, be good reasons for prioritizing non-viable embryos as research material, for instance, because any moral worth accorded to them can at most be symbolic,

whereas viable embryos may be understood as having an intrinsic value based on their presumed (active) potential. Without such prioritization, the standard application of the subsidiarity principle would impede the creation of embryos (including the creation of ELS that qualify as such) for research purposes that can be pursued with supernumerary IVF embryos. For those who argue that (non-)viability is merely contingent upon the availability of the right external triggers, the case for prioritizing research with non-viable embryos (and ELS) is weakened. Here, the development of (the currently non-viable) ELS reveals again the indeterminateness of present normative frameworks at a crucial point.

CONCLUSION

PSC-based ELS recapitulate various aspects of early mouse and human development, including the formation of the epiblast, trophoblast and amniotic cavity, as well as gastrulation-like events. Overall, ELS remain a promising platform for elucidating critical processes during mammalian embryogenesis, potentially delivering greater flexibility and higher-throughput compared to studies involving fertilization-based embryos. Given these benefits, it is reasonable to expect that the quality of ELS will rapidly improve, allowing experts to mimic aspects of embryogenesis ever more accurately. Where human ELS are concerned, this may prove especially favorable in evading the ethical and legal restraints imposed on the use and/or creation of human fertilization-based embryos for research. At the same time, the more ELS succeed in replicating natural development, the more urgent it becomes to consider the extent to which their use may raise (part of the) moral concerns typical of human embryo research. In terms of agenda setting for ethical refection and societal debate, the following issues stand out.

A first issue concerns the robustness of present embryo definitions underlying embryo protection regulations. To be adequate, such definitions should not a priori rule out the possibility of ELS qualifying as human embryos, nor should their applicability comprise epistemological uncertainty. In this regard, defining the embryo in terms of its ability to initiate embryogenesis, thus remaining closest to what scientific practice regards as embryos, seems the more promising alternative because it avoids limiting the scope for moral debate on a conceptual level.

Secondly, it needs to be clarified whether the concept of active potentiality can survive as a basis for according moral standing in times where embryos can be assembled and disassembled as cellular building kits. If the concept must be abandoned also for fertilization (or SCNT)-based embryos, present normative frameworks for embryo protection would require much rethinking. However, if active potentiality remains an important cornerstone of the framework, programmed non-viability would keep ELS,

and fertilization (or SCNT)-based embryos subjected to similar programming, in a different moral league, where symbolic rather than intrinsic value is all that possibly counts. This is directly important for determining whether such ELS (as well as non-viable embryos generally) should be given priority as research material under the principle of subsidiarity.

A final issue urgently requiring ethical reflection and debate is the 14-day limit as *terminus ad quem* of human embryo research. Where understood as referring to mere developmental duration, the rule cannot be meaningfully applied to ELS because their developmental stage at the first day of culture can correspond to that of a several days old fertilization (or SCNT)-based embryo. Instead of a time limit, what is needed is an account of morally relevant features, the emergence of which would render research with human embryos (even when non-viable) problematic. Whether PS formation would qualify as such, is at least not obvious. Here again, what complicates the necessary debate is the developmental fluidity of ELS, not only allowing the bypass of certain stages all together but also enabling certain features to be switched 'on' and 'off'.

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CHAPTER 3

The closer the knit, the tighter the fit: conceptual and ethical issues of human embryo modelling

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ABSTRACT

The recent generation of human blastocyst-like structures mark an important further step in the modelling of human embryogenesis. Unlike fertilization-derived ('natural') human embryos, 'embryo-like structures' (ELS) can be created and modified *ad libitum*, thereby overcoming shortage of research material, enabling large-scale manufacturability, and expanding scientific possibilities. Most prominently, this new field provides the unprecedented opportunity to study human embryology in a bottom-up and decoupled manner. In addition to these technical benefits, ELS-research is thought to provide the normative advantage of circumventing the ethical sensitivities and legal restrictions of human embryo research. In this commentary, however, we caution that the closer we get to creating a 'perfect replica', the tighter its research use will (need to) be monitored. We show how this is complicated not just by the lack of a shared embryo definition, but also by the fact that some of these definitions may serve to avoid, rather than address, the ethical and legal questions raised by developments in ELS-research. Finally, we indicate that the ethical debate is not only about how ELS-research would fit existing normative frameworks, but also about whether these frameworks may need revisiting or expanding in light of their development.

KEY WORDS
Embryo-Like Structures; Human Blastoid; Ethics; Policy; Embryo Definitions; Embryo Research Legislation;

INTRODUCTION

Recent publications reporting the generation of human blastocyst-like structures (also known as 'blastoids'; **Liu et al. 2021; Yu et al. 2021**) mark an important further step in the modelling of human embryogenesis. This emerging field of research uses advanced (stem) cell technologies and culture systems to enable new insights into early human development and reproductive health. Moreover, it promises to do so in a way that overcomes current limitations on human embryo research. Unlike human embryos, stem cell-based 'embryo-like structures' (ELS) – such as 'blastoids' or 'gastruloids' – can be created and modified *ad libitum*, enabling studies that require large numbers of genetically identical entities, while bypassing the need for oocyte donation. What is more, ELS-research provides a bottom-up approach to human embryology, which is not possible with fertilization-derived ('natural') embryos. In addition to overcoming shortages of research material and expanding scientific possibilities, the main benefit of ELS research presumably lies in its potential to circumvent the ethical sensitivities and legal restrictions associated with human embryo research.

ELS research as a 'Win-Win' policy

The destructive use of human embryos, even for important purposes, remains highly controversial due to conflicting views on the moral status of early human life. In jurisdictions where human embryo research is allowed, it is only permitted within 14 days post-fertilization (the so-called '14-day rule') and often only if conducted with surplus embryos. However, insofar as ELS are just models, these restrictions simply do not apply. It is therefore not surprising that furthering ELS research is widely regarded as a 'win–win' policy, promising scientific progress and its ensuing societal benefits, while avoiding the restrictions and burdens of human embryo research. The Dutch government, for instance, has launched a €14 million programme for research consortia on the advancement of human ELS explicitly with an eye to making human embryo research as redundant as possible.

This is of course assuming that ELS are and will remain just that: embryo models, rather than embryos. Where concerning 'non-integrated' ELS, such as present-day human gastruloids for instance, this is not really an issue. Although clearly promising tools for both fundamental and applied research (e.g. toxicity testing), they lack relevant cell types and have a limited developmental potential (**Moris et al. 2020)**. Indeed, for answering many specific research questions, ELS need not be 'perfect replicas' of human embryos in every respect. But with the human blastoids that were recently created, ELS-research has taken an important step forward in precisely that direction **(Zheng & Fu 2021)**. Despite their remaining limitations, these 'integrated' blastocyst-like models represent all the cell types needed for the development of both the fetus and supporting tissues. Still, the hurdles on the road to creating high-fidelity

human ELS remain considerable. As stressed in a recent review, important challenges include benchmarking these models against 'natural' embryos, on which (comparative) studies are lacking **(Posfai et al. 2021)**. The development of ELS that are functionally capable of replacing 'natural' embryos will therefore itself require parallel human embryo research (also beyond 14 days), which should serve as a sobering note for those counting on immediate benefits of the aspired 'win–win' policy.

Once these hurdles are overcome, it may become increasingly difficult to distinguish between the functional properties of ELS and those of 'natural' embryos. While this would support claims of sufficient similarity to replace embryos in research, it would also raise the question of how to ethically and legally distinguish between ELS that are (still) just models and ELS that should be regarded as (stem cell-derived) embryos. The paradox that emerges here is that the better these models become, the less useful they may be precisely as (embryo-replacing/saving) models **(Pereira Daoud et al. 2020)**. There is a tipping point beyond which greater similarity collapses into identity, and ELS research into human embryo research. Where precisely this tipping point lies is not a question that can be easily resolved. Whereas with animal ELS the ultimate test would be the birth of healthy and fertile offspring **(Posfai et al. 2021)**, this route is for obvious ethical reasons inaccessible where human ELS are concerned.

In order to maintain the benefits of embryo modelling over embryo research, it may thus be prudent to err on the side of safety and steer clear of attempts to create the 'perfect replica'—not because crossing into territory where ELS might be more than just models would be ethically problematic in itself, as some authors seem to suggest **(Moris et al. 2021)**, but rather because so doing would ultimately bring back the ethical and legal restrictions ELS research meant to circumvent, thereby also revoking debates about whether and how these restrictions should be revised. Of course, acknowledging that there is a limit to the envisaged 'win–win' policy does not detract from the value of developing ELS as a context for bottom-up and decoupled approaches to exploring principles of development.

Conceptual issues: the political use of embryo definitions

The fact that we lack a universal definition of what, for ethical and legal purposes, should count as a human embryo complicates matters even further. For researchers, it means that research with (particular 'types' of) human ELS—especially if improved—may be severely limited in some jurisdictions, while not requiring the same (or any) level of regulation in others **(Matthews & Morali 2020)**. For politicians, some definitions open up the possibility to have it both ways: benefiting from research with ELS (however perfected), while taking the moral high ground with regard to embryos.

The historical precedent for this is how countries that (like Spain) continue to define the embryo as the fusion of a human oocyte by a human sperm could proceed to ratify the Oviedo Convention—with its ban on creating research embryos—while still

allowing somatic cell nuclear transfer. The price, of course, was to deny that Dolly the cloned sheep originated from an embryo. Should it become possible to create offspring from perfected animal ELS, countries with fertilization-based qualifications may follow this precedent and maintain that, by definition, human ELS are not embryos, however perfected they may become.

Similar strategies are possible in jurisdictions that define the embryo exclusively in terms of its developmental potential (e.g. Belgium and the Netherlands). On this score, human embryos that—for whatever reason—are incapable of growing into a child are not embryos for legal purposes. Whereas in earlier debates commentators have called it a problem that this denies the very existence of non-viable human embryos, the Dutch government now seems to see this as an opportunity. Case in point being the aforementioned funding for ELS research, of which a quarter is destined for a consortium developing so-called 'non-viable IVG-embryos', i.e. embryos created through the fertilization of stem cell-derived gametes (in-vitro gametogenesis, or IVG) that have been pre-emptively genetically modified to ensure non- viability. The funding call refers to these as further 'embryo models' with the specific advantage of allowing research on fertilization and post-fertilization processes, stages too early to model with present-day ELS. The political motive is obvious: developing 'non-viable IVG- embryos' would allow the Netherlands to invest in research on early human development without having to lift its ban on research embryos, an issue that still strongly divides Dutch politics and society. Similarly, if scientists were to programme 'suicide genes' in ELS, these models would also fall outside the Dutch embryo definition, regardless of how perfected they are. Politically loaded definitions such as these are problematic insofar as they are used to avoid, rather than address, the ethical and legal questions raised by new developments in ELS research.

Ethical issues: potentiality and beyond

The human blastoids developed by Yu's and Liu's groups **(Liu et al. 2021; Yu et al. 2021)** underscore the urgency of reconsidering the moral bearing of the so-called 'potentiality argument'. Some scholars argue that the cellular convertibility demonstrated in ELS comes to show that the whole idea of an 'intrinsic' and 'active' potential is simply unfeasible **(Stier & Schoene-Seifert 2013)**. For these scholars, ELS research is evidence that developmental potential is entirely a matter of contingent factors that can be arbitrarily switched on or off. If proven correct, a cornerstone argument that has generally been taken to grant early human embryos special moral treatment is no longer available: the idea of the human embryo as autonomously capable of growing into a human being under the proper circumstances. From an ethical perspective, this would mean more room for human embryo research, including research beyond the 14 days that legislation currently allows.

But the case against potentiality remains an issue for further analysis and debate

(Hyun 2013), with some authors conversely arguing that ELS research may precisely demonstrate a stem cell capacity to initiate autonomous development under the right conditions **(Denker 2021)**. Supposing, for the sake of argument, that the potentiality argument withstands, two side-notes are still worth making. One, it is a misunderstanding that 'active' potentiality would entail 'full moral status'. In fact, the argument is perfectly compatible with the view that human embryos have only limited moral status and can, therefore, be used for research purposes under conditions of proportionality and subsidiarity. Two, many advocates of the argument have argued that 'active' potentiality presupposes numerical identity between the different stages of the developing organism **(Buckle 1990)**. This would entail that 'active' potentiality can only gain moral traction if natural twinning is no longer possible, meaning that only post-implantation stage embryos or ELS would qualify for protection on this basis, and blastocysts or blastoids would not **(Pereira Daoud et al. 2020)**.

Other ethical issues can be expected to emerge precisely with regard to ELS that are clearly not embryos and that, for that reason, would not be bound to the restrictions imposed on human embryo research (such as the 14-day limit). If these ELS are used to model human organogenesis, beating hearts or early brains may be regarded by society as especially sensitive, leading to discussions similar to those raised by brain organoids. Of note, brain cells are not replicated in present human gastruloids, but this may change with their further improvement. Even so, apart from the hypothetical concern that entities with (very) rudimentary brains could become sentient and feel pain, it is unclear why such issues should be regarded as categorical cut-off points for research. Whereas, in developing embryos, beating hearts and early brains might be regarded as markers of what the embryo is growing into, and thus merit some degree of symbolic value, no such argument is available where ELS are concerned that are clearly models, and not embryos **(Pereira Daoud et al. 2020)**.

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PART II

Refinement through Empirical Validation

CHAPTER 4

The ethics of stem cell-based human embryo-like structures: a focus group study on the perspectives of Dutch professionals and lay citizens

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CHAPTER 5

Dutch perspectives on the conceptual and moral qualification of human embryo-like structures: a qualitative study

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ABSTRACT

The number of publications on the governance of research with human embryo-like structures (hELS), i.e., 3D aggregates of human (induced) pluripotent stem cells made to model early human development, is growing rapidly. Public involvement is called for in many of these publications, but studies on public perspectives towards this emerging field remain lacking due to its novelty. To reduce the gap in the literature and contribute to the ongoing scholarly debate, we conducted interviews with Dutch lay citizens, health law and health care professionals, and interviewees reasoning from prominent worldviews in the Netherlands. This article reports on these participants' views about the conceptual and moral qualification of hELS. With regard to the conceptual qualification of hELS, participants believed it should provide a shorthand for their (dis) similarity to human embryos, but differences remained with regard to the features upon which this (dis)similarity should be based. With regard to the moral qualification of hELS, participants believed this should depend on whether or not hELS possessed the features they considered morally relevant, among which those associated with sentience and a potential for continuous human development. Taken together, these findings align well with the arguments and positions traditionally found in related ethical debates and the recently proposed recommendations for the governance of research with hELS specifically. As such, they may also help allay concerns about lay publics not being able to meaningfully participate in debates about the ethical ramifications of (novel) scientific developments.

INTRODUCTION

The growing interest in human embryo-like structures (hELS), i.e., 3D aggregates of human (induced) pluripotent stem cells, is driven by the belief that they come sufficiently close to human embryos to complement their research use, while remaining sufficiently different from them to circumvent the technical, legal, and ethical sensitivities of human embryo research **(Rivron & Fu 2021; Rossant & Tam 2021; Posfai et al. 2021; Mummery & Anthony 2021)**. Despite the growing stream of academic publications on the science and governance of this emerging field **(Hyun et al. 2020; Pereira Daoud et al. 2020; Sawai et al. 2020; Clark et al. 2021; Matthews et al. 2021)**, little is known about public perspectives on the conceptual and moral qualifications of hELS, and on what this should entail for the use of these structures in research. Our empirical study aims to reduce this gap in the literature and to contribute to the ongoing scholarly debate by probing these issues through the lenses of lay citizens, health law and health ethics professionals, and individuals reasoning from prominent worldviews in the Netherlands.

In this paper, we first describe our methods, including the sample, setting, and analysis of the research data, after which we report on the findings pertaining to the participants' conceptual and moral qualification of hELS, respectively. This division between 'concepts' and 'morals' is merely pragmatic: we are of course aware of the interconnectedness between the descriptive and the normative, and by no means wish to purport that the two can be strictly untangled. In the section "Discussion", we bring these results together and illuminate the areas of common ground and those that prompt debate. We conclude by relating these findings to recent guidelines and pinpointing issues in need of further enquiry.

METHODS

This paper is part of a larger study in which we explore the range of professional and lay perspectives on the creation and research use of hELS. Data were collected between August 2020 and March 2021 in the Netherlands. The full data set consisted of three focus group interviews with a cross section of the Dutch public, one focus group with health law and health ethics professionals, and five in-depth interviews with individual representatives of prominent worldviews in the Netherlands **(CBS 2020)** (for the full research sample, see **Tables I–III**). In this article, we report on the findings pertaining to the participants' conceptual and moral qualification of hELS.

Research sample and setting

The four focus group interviews (N = 33) were held in August and September 2020 and

lasted two hours on average. Three of these were conducted with lay citizens. The lay citizens in the pilot focus group (FG-PILOT, n = 5) were selected from the network of APD based on the demographic characteristics (sex, age, education level) of the Dutch population and invited personally. For the other two focus groups with lay citizens (FG-LAY1, n = 10 and FG-LAY2, n = 11), we hired a recruitment agency to select a representative cross-section of the Dutch population. Participants in these groups received a small (€50,-) financial compensation. The fourth focus group interview (FG-Professionals, n = 7) was conducted with health law and health ethics specialists selected from the networks of the authors, based on the participants' affinity with debates on the ethical, legal, and societal implications of comparable emerging biotechnologies.

Considering that perspectives on human embryo research can be strongly intertwined with (non-)religious worldviews, and assuming that the same may hold true for research with hELS, five in-depth interviews were conducted with interviewees known to reason from worldviews prominent in the Netherlands (Catholicism, Protestantism, Judaism, Islam and Humanism) and familiar with related bioethical debates. The aim of these interviews was to supplement the data gathered in the focus groups. These interviews were held between August 2020 and March 2021, lasted one and a half hour on average, and were held online via BlueJeans.

Data collection and analysis

For consistency, focus group and individual participants received the same invitational letter and set of semi-structured questions. These open-ended questions (see Supplementary Information) were formulated in ways that allowed participants to expand on topics they personally considered important whilst still probing the ethical and policy issues we had previously found to be in need of further enquiry **(Pereira Daoud et al. 2020)**.

The interviews, held in Dutch and audio recorded, were transcribed verbatim and pseudonymized for thematic analysis. Interviews with participants reasoning from prominent worldviews were additionally summarized and sent to the respective participants for approval. Considering the explorative nature of our study, the first step in our thematic analysis was to create open codes that tentatively labelled important passages. These codes were created in Atlas.ti 8 by APD. After the full list of open codes was validated by WD through a randomized sampling method, APD clustered them in a mind map based on the questions to which they were related. The resulting clusters were evaluated and adapted in meetings with the research team until higher order themes could be consistently agreed upon by all members. The thematic data analysis resulted in four themes, two of which we discuss below[1].

1 The two remnant themes are reported in a separate manuscript.

RESULTS

The conceptual qualification of hELS: origins and potentialities

An important topic in relation to hELS is what language we should use to refer to them. At present, various general, developmental time-based, and cell-based names **(Matthews et al. 2021)** co-exist in an effort to denote these structures and the particular differences between them. In what follows, we elaborate on the participants' perceptions of prominent umbrella terms and the features that they considered key in developing appropriate terminology for hELS.

Focus group interviews

When asked which of two general terms—i.e., 'synthetic embryos' or 'embryo-like structures'—the participants preferred to denote hELS, neither term was overwhelmingly favoured. Both were considered misleading, albeit for opposite reasons. The term 'synthetic' was viewed as delusive because it 'dehumanized' the models in question. For several participants, 'synthetic' invoked the impression that "you could create [hELS] from stuff you can find on the kitchen table, so to speak", thus making it "very unclear that it consists of human components." By contrast, the term 'embryo-like' was viewed as misleading because it allegedly prematurely prompted people to "immediately think, 'oh, it is a human being; oh, it is an embryo'."

Participants were noticeably mindful of the impact these perceived connotations could have in steering public opinion. They worried particularly that vague, general terms may pre-empt the ability to form an opinion. One of the professionals, for instance,

> "… struggle[d] a lot with the word 'embryo-like structure' because all kinds of things could fall under it … and with that, it becomes a black box [to determine], what exactly are scientists doing in the lab? And how can we societally and democratically find something of it? With those kind of container terms, that is all covered up."

Bearing these connotations in mind, we asked lay and professional groups to consider how they would define hELS vis-à-vis human embryos. Here, two contrasting attributes—origins and potentialities—were key, with neither group having a clear preference for one of these attributes.

On the one hand, were participants claiming that appropriate terminology should reflect the origins of hELS; i.e., how they came into being. As argued by two professionals, for instance, "I would think [that] an embryo—actually, my first intuition is then that a sperm cell and an egg cell got together. To me, that is then an embryo", which would imply that hELS are categorically different from embryos. In lay groups, this view was even more pronounced. Here, several lay participants argued that an embryo "is

created from fertilization and that [a hELS] is created from stem cells—and there is a difference", or that the difference between a "'natural' embryo, [which originates] from two unique gene sets, or a clone-embryo" must be clear.

On the other hand, were participants that believed appropriate nomenclature should denote the expected potentialities of hELS; i.e., that it should reflect their "viability" or "potential to grow into a human being". As argued by professionals, "… my gut-feeling would be that [an embryo] is a human being in the making", and "the moment that you can actually create something with those embryo-like structures—an entity that, if implanted, could [grow into] a human being—that is, at least morally, … very relevant." Similarly, lay participants argued, "… if you think of an embryo …, then [you think of] the creation of a baby, so that's what you assume, regardless of how small it is", and "if [a hELS] could potentially go through [embryonic] development, then it is an embryo".

The difficulty of reaching consensus on whether hELS qualify as (non-)embryos seemed to be—at least partly—the consequence of current embryo definitions, which one professional succinctly summarized as

> "… a tablecloth that is too small to cover the table. If we pull it in the direction of fertilization, we'll have a table not fully covered because [it leaves us with] cloned structures. If we draw it towards … the potential to develop into a human being, I think we also have a problem because … then it is very difficult to speak of non-viable embryos."

Individual Interviews

Most interviewees had no strong terminological preference either. In fact, from those reasoning from religious worldviews, the Protestant interviewee was the only one to explicitly prefer 'embryo-like' over 'synthetic'. Like some focus group participants, this interviewee felt that 'synthetic' may be too 'dehumanizing' in the sense that it can evoke the idea that these structures are 'not real' and, therefore, be taken to prematurely imply that they cannot matter morally. Simultaneously, it may be too 'anthropomorphic' in the sense that it could be taken to mean that these structures are embryos in the morally relevant ways—albeit embryos that were created through artificial means.

Terminology that would prematurely imply the latter conclusion was deemed inappropriate by all interviewees reasoning from religious worldviews. Driving these intuitions was the view that the key denominator between embryos and non-embryos should be their potential to develop into a foetus and then into a child, which was understood as denoting the continuous development of the human being that the embryo already is. This is also why these interviewees explicitly referred to human embryos as 'human beings in the making', 'human beings embodying themselves', or as 'specimen of their kind'.

By contrast, the interviewee reasoning from a Humanist perspective was the only

one to lean towards defining the human embryo in terms of how it usually comes to be—i.e., the process of fertilization. hELS that come so close to fertilization-derived embryos that they are functionally indistinguishable from them could then be denoted as 'artificial embryos', whereas those that come less close might be referred to as 'embryo-like' structures. This interviewee stressed the ethical importance of holding on to conceptual ambiguities as arising from technological developments such as those in this field, noting that the resulting moral ambivalence is something to be faced rather than avoided.

The moral qualification of hELS: moral status and beyond

Another currently discussed issue in relation to hELS is whether they can provide a morally preferable alternative to research with human embryos **(Rivron et al. 2018; Wilger 2019; Nicolas et al. 2021; Rossant & Tam 2021; Posfai et al. 2021; Moris et al. 2021)**. To probe the participants' views on this topic, we developed two practical exercises, which we discuss separately below.

The moral protection due to human and non-human organisms: exercise I.

The first exercise asked participants to place ten distinct living organisms in pecking order; the higher their rank, the more protection they should be afforded. These organisms were (in no particular order): a human zygote, a human embryo (of roughly 8 weeks), a human foetus (of roughly 24 weeks), a mouse, a chimpanzee, a toddler, an adult person, a fish, a tree, and a human gastruloid. The 'human gastruloid' was included as a concrete example of ELS due to it being one of the first types created from human stem cells at the time. The further particularities of 'gastruloids'—such as their lack of extraembryonic cells and, therefore, incapability of continuing integrated human development—were not further discussed, thus purposefully leaving open the question whether or not hELS could (come to) have the potential to develop into human beings.

<u>Focus group interviews</u>

Most participants preferred to group several organisms together, thereby creating threshold-based rankings. In these groups, the adult and toddler structurally shared first place, after which the foetus often immediately followed. The mouse and fish were often also grouped together, generally taking a middle-low position in the rankings. At the bottom of most rankings, were the tree and gastruloid, though the gastruloid was more often viewed as being due greater protection than the tree. In fact, for two lay participants, the gastruloid was due the same protection as any other human organism, thus sharing first place with the adult, toddler, foetus, embryo, and zygote. The chimpanzee, embryo, and zygote were clearly borderline cases, with most participants struggling to position them in the ranking (between high and middle-high), though

more often conferring greater protection to the chimpanzee, then to the embryo, and then to the zygote, respectively.

A recurring consideration underlying these rankings was that sentient beings deserve at least some protection. As explained by one of the lay participants:

> "I think that ethics depends on the [capacity to feel]. We would not need to … have a discussion about bricks. Because bricks are bricks, right? But the moment a living entity can feel, then you can start to wonder how you should treat it."

For some participants, a 'capacity to feel' (or: sentience) was the single most important basis for affording protection. Others came up with more elaborate distinctions between sentient beings, distinguishing between those with a mere physiological capacity to feel pain, and those capable of more complex forms of self-awareness. As continued by the participant quoted above, for instance:

> "You know, another criterion is whether the being in question … is conscious of its situation. … I think a chimpanzee is more aware of his environment than a fish …. So the chimpanzee can estimate for himself whether he is in a situation in which he is happy or in which he suffers."

The question whether the zygote, gastruloid, and embryo met this criterion—even if only in a rudimentary sense—sparked debate in lay groups. For some, the early developmental stage of the zygote and hELS was perceived as evidence that they cannot be sentient at all, and therefore reason to confer them the least protection. From this, it followed that the (degree of) protection afforded to embryos would equally depend on their 'capacity to feel', which prompted discussions about neural development and brain activity at this stage in embryogenesis.

Simultaneously, the fact that several participants conferred greater protection to embryos and foetuses (and, in some cases, to the zygote and gastruloid) than to most (and, occasionally, all) non-human animals, reveals that considerations other than sentience must also have been at play. Some reasoned, for instance, that human organisms should be given precedence over non-human organisms simply because they are human:

> "It may sound very blunt, but we're talking about an animal or a human being. … I think that… Well, it is human. While actually precisely the same worth—in terms of life— [is at stake], eventually it is the last one standing that wins."

Others reasoned that the relatively higher protection afforded to embryos and foetuses had to do with their special potential toward further human development:

> "An embryo develops—in the ideal situation …—into a human being. And then you have [to consider] the worthiness [of the embryo] at present, but you also have [to consider] the worthiness [of the embryo] in terms of [its] potential."

For one of these participants—who, under explicit reference to that 'special potential', attributed maximum protection to all human beings—the question whether that protection should extend to hELS would thus depend

> "… on whether you can define it as an embryo or not. I think [that] if you can define it as an embryo, or at least as 'embryo-like', then I think it has as much worth, intrinsically, as a human adult."

While this participant argued that, if hELS had this potential, they would be due (full) protection throughout, others felt it did make a difference that it was only the early stages, as compared, for instance, to a foetus.

Finally, participants also suggested that hELS deserve a lower ranking in view of their explicit research purpose. For instance: "That embryo-like structure has been created in a lab somewhere. With the purpose, I presume, to do research. And then I find that purpose more important than the purpose of the embryo to [grow into a human being]." Connected with this was their artificiality and easy replaceability:

> "… at the bottom is the embryo-like structure. Because, for me, it comes from a laboratory and is, as it were, subservient to what we want to know and what we can do with it, and so on. So it's really different for me. … That [hELS] we can basically just throw in the trash. … I have … instinctively much more respect for that oocyte and that embryo than for that embryo-like structure."

When we asked whether this participant would think differently if hELS could grow into human beings, the answer was still 'no' because the resulting clone would be an artefact: "… even then, it would not be a real human being in my view, because it originates from an existing DNA". Similarly, a professional who accorded the highest ranking to "everything that is or can become a human being" did not reason that hELS— if capable of growing into a human being—should be on the same level. Instead, the professional placed such hELS still "somewhat … lower because they are more artificial".

Individual Interviews

The interviewees reasoning from a religious worldview conferred the highest degree of protection to all (potential) human beings, i.e., the adult, toddler, foetus, embryo and zygote. Next came the fauna, with the chimpanzee ranking highest amongst the nonhuman animals. For these interviewees, the ranking of the gastruloid was conditional on its potential to develop into a human being. If we were to assume that gastruloids have this potential, then they would all rank it as a human being and confer it maximum protection. If we were to assume that gastruloids do not have this potential, then their ranking would be lower. Whereas interviewees reasoning from a Catholic, Protestant, and Jewish perspective would place it below the flora, and thus confer it the least degree of protection, the interviewee reasoning from an Islamic perspective would rank it between the human and animal categories, thus conferring it a middle-high degree of protection.

Of note, the interviewee reasoning from an Islamic perspective argued that, even if hELS could one day acquire a capacity for further development, this would still be an artificially acquired capacity, rather than an autonomous or inherent one. From this, it followed that research with hELS would be preferable over research with embryos—even if both were capable of developing further. The interviewees reasoning from a Christian and Jewish perspective did not discount the possibility of hELS one day acquiring the autonomous or inherent potential to develop into (or: as) a human being. Instead, they stressed that, with science moving forward, the problem is that one cannot know with certainty—nor establish in ethically acceptable ways—whether or not improved variants of hELS may have this potential. When asked their thoughts on the possibility of averting this uncertainty by programming so-called 'suicide genes' so as to make hELS incapable of further human development, all three interviewees—although most emphatically those reasoning from a Christian perspective—argued this would be paradoxical: would suicide genes really prevent hELS from acquiring the relevant potential, or would they merely frustrate it? For those reasoning from a Christian background, the only way to deal with this epistemological uncertainty was therefore to err on the side of safety and simply not create hELS.

The interviewee reasoning from a Humanist perspective was the only one to diverge from the '(potential) humanity > fauna > flora' outline. In her/his ranking, the adult, toddler and chimpanzee were afforded maximum protection, after which followed a separate category for the foetus. In third place, came the fish, mouse, and tree. Finally, at the bottom, and due the least protection, were the embryo, zygote, and gastruloid. Though this interviewee struggled to pinpoint the exact reasons for this ranking, an important consideration seemed to be that the "more complex and communicating" the organism, the greater the protection they should be afforded.

The moral protection due to early human organisms: exercise II.

In the second exercise, we asked participants to evaluate three hypothetical research proposals as if they were members of a hospital's ethics committee. These proposals involved research with surplus human embryos, human embryos specially created for research ('research' embryos)[2], and hELS. The aim of these (hypothetical) scenarios was to enquire the participants' perspectives on embryo research and ensuing implications for research with hELS. The research proposals were imagined to meet the relevant legal requirements, to be methodologically sound, and to potentially provide relevant new insights to the improvement of *in vitro* fertilization (IVF).

Focus group interviews

The hypothetical research proposal with surplus embryos did not spark any significant debate among professionals, who argued not to see the problem of such research if conducted under current legal conditions. In lay groups, however, there was debate. For many, surplus human embryo research was perfectly acceptable if the proposal were sound and seriously considered, the idea being that "the benefits of [surplus embryo] research prevail" for several reasons. Arguments were that surplus embryos "are already here anyway, so … if we already have them, it's better to do something useful with them than to just throw them away", that embryos at these stages "are not yet so far developed that you could say you are really harming them", and that research aimed at improving IVF was considered a worthy end. But in every lay group, there was also at least one participant conveying the view that even though surplus embryo research "is [legally] allowed, … I don't think it should be". In fact, one of these participants was

> "… actually a bit shocked that there even are surplus embryos at all. And that they are then destroyed. … I get that it is reasoned that, if they are to be destroyed anyway, then so be it; do research with it. But I would actually prefer that there aren't any spare embryos to destroy or do research with at all."

For this participant, the main reason to reject surplus embryo research was the view that "there is an intrinsic value to embryos, whether they are surplus or not", and that recognizing that value would mean recognizing that they are "no different from a terminally ill person … that you have to care for until the end, with everything you can." A more widely shared argument to object to surplus embryo research, however, was

2 Note that the Dutch Embryo Act presently prohibits the creation of human embryos for research purposes. At the same time, the ban is also the subject of current societal debate. For the purpose of our study, participants were therefore asked to imagine a scenario in which, as a result of this societal debate, the ban would have been lifted and research with 'research (human) embryos' would have been legally allowed under certain conditions (see Supplementary Information).

the thought that "there is a very clear price for 'manufacturability'", and that we should steer clear from attempts "to play God and [make things] better and more perfect but [only] to our [own] advantage, not [to the advantage] of the whole picture, so to speak", which would include accepting life's imperfections, such as infertility.

The proposal with research embryos sparked debate across all groups. In the focus group with professionals, the main issue of discussion was under what conditions it could be justified to create embryos for research. Prominent considerations were the aims of research, which had to be "very important", and the alternatives available, given that research with research embryos should only be considered if its aims "cannot be obtained in any other [morally less controversial] way", including the use of surplus embryos. Even so, one of the professionals struggled with the acceptability of creating embryos for research: "as an academic, I'd say yes, actually it should be possible, for example, for the improvement of IVF. But if I look at my ranking [in the previous exercise], then it actually doesn't feel quite right." Lay participants were also visibly more uncomfortable with this proposal than with the previous one. Whereas some argued that "whether they're surplus embryos or whether you [specially] create them yourself, comes down to nearly the same thing", most agreed that there is still an important difference. As summarized by one of these participants:

> "I wouldn't agree [with this proposal]. I feel like [surplus] embryos were created for IVF, so those were already made in case [the IVF-treatment] didn't work …. [But] when [embryos] are specially created, [they are] created to be killed. And I don't know, I think … that's the difference for me. They are made for a different purpose."

For several participants, this difference was big enough to object to the creation of research embryos. Others felt that certain research aims may still be important enough to justify it if, "with the results of that research, you [could] help people [more] than you could inflict harm on those embryos".

The participants' views on the permissibility of research with hELS depended on the features hELS possessed, among which most prominently those associated with the capacity toward further human development and the capacity to feel. Lack of developmental potential, for instance, was generally taken to mean that research with hELS should be given precedence over research with embryos. After all, as a professional stated, "the attractive thing about [hELS] seems to be that (…) they don't have that potential at all." But once hELS were conceived to have that potential, views diverged. For one of the lay participants, for instance, if hELS had a potential toward further human development, research with them would be tantamount to research with embryos and, on this participant's view, impermissible. Others agreed that if hELS were to have a developmental potential akin to embryos, their research use would

indeed become more contentious, but for different reasons. One of the professionals, for example, preferred that research were conducted with research embryos over (viable) hELS due to the concern that the latter may open opportunities for misuse, such as the possibility "to create clones." This prompted a fellow professional to argue that even if hELS were to have the developmental potential of embryos, there would still be good reasons to prioritize their research use:

> "Look, if you can just create [hELS] from a little bit of material you already have, then you don't have to ask me which has my preference. If the risks and the moral protection [that should be afforded] are the same … I still think preference should be given to [hELS] because you do not need oocyte donors [to create them]."

Another reason to prefer research with hELS over human embryos—even if both were conceived to be viable—was their 'artificial' origin. This reasoning was especially perceptible in lay groups, with several participants arguing that they intuitively felt hELS were due less protection than embryos precisely because they are merely "put together", "artificial" or "something out of a lab", meaning that their research use could thus be "subservient to what we want to know and what we can do with it".

For several—though again especially lay—participants, these considerations implied that research with hELS should be allowed until stages preceding the development of the organismal features they thought were morally relevant, among which most prominently those associated with sentience. In one of the lay groups, for example, this was taken to mean that research with hELS could continue "up to a few weeks. ... Well, what had we just said …? Four weeks? Eight weeks? [Up to the development of] the nervous system, [up to] that [point]". Another feature was a beating human heart:

> "Well, if it is an embryo-like structure, [and] if it is not the case that the heart starts beating on the 22nd day, then you can do research for a longer [period of time]. ... But the moment the heart develops, I say 'until here and no further'."

Individual interviews

Notably, all five interviewees were hesitant about human embryo research. For those reasoning from religious worldviews, this had to do with the embryo's moral status. This was especially clear in the interviews with Catholic and Protestant interviewees, according to whom it is because human embryos are due full moral status from conception that they should never be treated as mere means, but always also as ends in themselves. This meant that research with both surplus and research embryos is categorically wrong. As the Protestant interviewee explained, the morally right

approach would be to create no more embryos than can be transferred in one IVF-treatment cycle. Since that is not how IVF is normally done, this interviewee felt that the best thing to do with surplus human embryos would be to "either offer them for adoption by couples who cannot generate embryos of their own, or just let them die. … If there is nothing you can do for a human being, you let it die. But you're not going to use it as experimental material, [just like you would] not use someone who is dying as experimental material."

The interviewees reasoning from Jewish and Islamic perspectives were not thrilled about surplus embryo research either, but they did consider it justifiable under certain conditions. This more liberal stance was based on the relatively low moral status that these perspectives confer to human embryos at stages preceding ensoulment—and the even lower status they confer to embryos ex utero. In particular, it was because of this relatively low status, combined with the utility that surplus embryo research could have for IVF or other medically important purposes, that the proposal was considered proportional. The creation of embryos for strictly instrumental purposes was considered by both interviewees as illegitimate, however. As explained by the interviewee reasoning from an Islamic perspective, the problem here is not that "… you're killing someone …. The specially created embryo does not have the moral status of a human being. But it does have a status—a moral status—that must be respected."

The interviewee reasoning from a Humanist worldview was also clearly hesitant about research with both embryos and hELS, albeit for very different reasons. Here, the predominant concern was that we might not be wise enough to deal with the powerful knowledge that this type of research could provide. For research with hELS particularly, this concern became especially pressing if imagined to be used reproductively and in combination with other recent technological advancements—such as CRISPR-Cas technology:

> "I do not mean to say that there can never be a good reason to do this [type of research] anyway. … But I have the strong feeling that we have to be very cautious [not] to build too much knowledge on this area because we're also building artificial wombs, we are also building ever more accurate gene [editing] tools… We are working with so much information and genetic technology that, at some point, we really do have a Brave New World."

By contrast, interviewees reasoning from Catholic, Protestant, and Jewish worldviews were notably receptive of research with hELS as long as they do not have the ('active') potential of human embryos. In that case, hELS were conferred at most extrinsic moral value and their research use was considered acceptable even beyond the emergence of—what several focus group participants considered—morally relevant features

(e.g., heartbeat or early brain development), which, for these interviewees, only mattered intrinsically if they indicated 'a human being in embodiment'. Although the interviewee reasoning from an Islamic perspective agreed that research with hELS that lack developmental potential would raise fewer ethical challenges than research with human embryos, it was also noted that they would still have "a special value because [the stem cells from which they are created] come from a human being. … So, if embryo-like structures arise from human material, then they are important."

DISCUSSION

In the first theme, consensus was found in the view that appropriate terminology for (specific types of) hELS should provide a shorthand for their (dis)similarity to human embryos. The contrast in the adjectives participants used to distinguish between hELS and embryos—e.g., 'artificial', 'synthetic', or 'embryo-like' structures versus 'real', 'natural,' or 'actual' embryos—suggests as much. The problem was reaching an agreement on the terms that could reflect this (dis)similarity effectively. For instance, whereas certain terms (e.g., 'synthetic') were perceived as inadequate for having normative connotations that could prematurely define the scope of public debate, others (e.g., 'embryo-like') were perceived as being so vague that they would prevent the ability to form a normative opinion altogether. These results suggest that too abstract terminology may indeed have little (or: too much) meaning to non-scientists **(Sturmey 2021)**, which echoes the growing scholarly emphasis on improving naming conventions for hELS **(Matthews et al. 2021; Rossant & Tam 2021)**. Prompting these issues, however, were more fundamental questions about the criteria upon which embryo definitions should be based. Prominent candidates were 'fertilization' and 'a potential to develop into a human being'. The fact that these questions arose not only in view of hELS but also in view of human embryos corroborates the agenda-setting input previously set forth with regard to the indeterminacy of traditional embryo definitions and the urgency to reconsider those **(Pereira Daoud et al. 2020)**.

The second theme consisted of two exercises, both of which were intended to understand how our participants thought about the protection that should be afforded to hELS. The first exercise asked participants to rank organisms of different kinds in order of importance. For the focus group participants and interviewee reasoning from a non-religious worldview this exercise was not easy. For most lay participants, it was not even something they had thought about before. It is therefore noteworthy that their rankings and considerations were very similar to those of the participants in the focus group with professionals, for whom at least the ethics of human embryo research was familiar territory. In these groups, non-human beings were generally ranked below human beings—at least, below human individuals that have been (the adult and child)

or technically could be (the 24-week-old foetus) born. Although a few lay participants relied on an unvarnished 'speciecist' reasoning, most others sought morally more substantive reasons for conferring greater protection to these over other organisms. Two chief considerations were: (1) 'are these organisms capable of feeling pain?', and (2) 'are these organisms capable of more complex forms of self-awareness?'

Considerations of this kind are prominent in traditional ethical debates about moral status, and map especially well onto the distinction McMahan draws between the morality of interests and the morality of respect **(McMahan 2002)**. Essentially, while the morality of interests applies to any being whose interests can be thwarted (among which non-human organisms), the morality of respect applies only to persons, i.e., beings "on an equal footing with ourselves" **(McMahan 2002)**. Although personhood is too philosophically complex to discuss in depth here **(Goodman 1998)**, it is commonly associated with more sophisticated capacities, such as "moral agency, autonomy, the capacity for intentional action, rationality, self-awareness, sociability, and linguistic ability" **(DeGrazia 2008)**. It is by virtue of these capacities that persons command respect, and it is by virtue of that respect that they should never be treated as mere means (instrumentalised), but always as ends in themselves. Organisms that fall below what McMahan refers to as 'the person threshold', however, may be justifiably used instrumentally, albeit under conditions of proportionality and subsidiarity **(Jans et al. 2018)**.

The (relatively low) protection many afforded to early forms of human life—i.e., the embryo, zygote and hELS—seemed to stem exclusively from considerations pertaining to the morality of interests. To these participants, the greater protection afforded to embryos than to zygotes was explicitly grounded in the idea that whereas embryos can be sentient, and thus have some interests that can be thwarted, zygotes certainly cannot. The same reasoning often also applied to hELS. If participants assumed hELS were incapable of feeling pain, most placed them at the bottom of their rankings. But as the second exercise later clarified, in the—admittedly very unlikely—event that hELS could become sentient, participants would confer them (much) greater protection from then on. Sometimes, a protection high enough to halt their use in research even if they clearly lacked any real developmental potential. For these participants, as well as for the interviewee reasoning from a non-religious worldview, considerations pertaining to the morality of respect were thus apparently not viewed as providing a suitable basis for protecting early (forms of) human life. Here, the underlying idea presumably is that the capacities associated with personhood cannot yet exist at these early stages, and that the protection afforded to zygotes, hELS, embryos, and even more fully developed foetuses, cannot stem from the respect due to persons. Hence, the only kind of considerations that could matter for these organisms' (intrinsic) moral status are those pertaining to the morality of interests.

At the same time, several other focus group participants and all individual

interviewees reasoning from religious worldviews placed early forms of human life very high in—or even at the very top of—their rankings. These rankings were based on 'arguments from potential', according to which early human beings are potential persons, if not persons with potential. As McMahan explains, to argue that early forms of human life are potential persons is basically to say that personhood is "a phase sortal—that is, a predicate that may apply to us only during a certain phase, or certain phases, of our existence" **(McMahan 2002)**. By contrast, to argue that early forms of human life are persons with potential, is to argue that all human beings are essentially persons, even if their personhood is only "latently or even occultly present" **(McMahan 2002)**. Those who placed forms of human life between high and middle-high positions but nevertheless discerned between the protection afforded to organisms at different stages, seemed to hold the view of potential persons. On this view, both the continuity (i.e., the developing entity is predisposed to become a person) and the discontinuity (i.e., the developing entity is not a person yet) matter morally. On the one hand, the fact that early forms of human life are not yet persons implies that the morality of respect cannot yet apply to them. On the other hand, the fact that they—unlike other (non-human) organisms—have a predisposition toward personhood provides an additional reason to protect them. The gradually increasing degree of protection these participants afforded to developing human beings at different developmental stages—conferring greater protection to foetuses than to embryos, and greater protection to embryos than to zygotes, even though all three are 'potential persons'—aligns well with this reading of potentiality. By contrast, those who placed all forms of human life at the top of their rankings without differentiating between them, seemingly view early human organisms as persons with potential. In this reading, personhood is an uninterrupted continuum: early forms of human life are essentially persons—albeit 'incomplete' ones, or persons in the process of becoming—and therefore stand 'on an equal moral footing with ourselves'. On these participants' view, it thus makes little sense to distinguish between the degrees of protection afforded to embryos and foetuses: both already are persons and therefore due equal respect. This reading was echoed by a (small) number of focus group participants, but much more pronounced in interviews with respondents reasoning from religious worldviews, which confirmed what we already knew about the moral status due human beings (with potential) on these views **(Walters 2004; Schenker 2005; Kerridge et al. 2010; Neaves 2017)**. Whereas the interviewees reasoning from Christian worldviews argued that it cannot be ruled out that personhood begins at conception, and therefore assumed the precautionary stance that the morality of respect would also apply to zygotes, the interviewees reasoning from a Jewish and Islamic perspective argued it begins at later stages (starting from the ensoulment, at day 40 and 120 in development, respectively), meaning that the morality of respect can only apply to human organisms past those stages.

Regardless of one's reading of personhood, the (gradually increasing or full)

protection participants' conferred human embryos based on their potential to become (or develop as) persons, would only extend to hELS if they too have that potential. For this potential to matter intrinsically, it must involve more than mere possibility. It must involve what the ethical literature denotes as 'active potentiality', i.e., an inherent and autonomously driven predisposition toward personhood **(Buckle 1990; Reichlin 1997; Denker 2006, 2021)**. The suggestion that any uncertainty about hELS acquiring such a potential could be avoided by building in 'suicide genes' is reminiscent of the earlier proposal for 'altered nuclear transfer' as a supposedly morally non-problematic approach to creating a source for patient-specific human embryonic stem cells (hESCs). This proposal involved combining so-called 'therapeutic cloning' with a genetically engineered defect meant to ensure that the resulting 'entity' would be unable to implant and, therefore, lack the potential to grow into a human being **(Hurlbut 2005)**. When this idea was discussed in the United States' President's **Council on Bioethics (2004)**, Doerflinger, the secretary of the American bishops' conference, proved not to be convinced by this strategy for the same reason brought up by some of our religious interviewees: for Doerflinger, the fate of human embryos that would have been modified to stagnate development after a certain stage was comparable with the limited life expectancy of persons known to be carrier of Huntington's disease. A further interesting observation from our results is that, for some participants, the 'artificiality' of hELS was taken to imply that these structures could not have the 'active' potential of human embryos—even if they too could produce a human being—and therefore reason to protect them less than human embryos at similar stages. This view was explicitly advocated by the interviewee reasoning from an Islamic worldview, for example. According to this interviewee, even if hELS had the potential to produce a human being, this potential would still be the result of external manipulation, rather than the result of an intrinsic and autonomously driven predisposition. That viewing the potential of (artificial) hELS as qualitatively different from the potential of (non-artificial) human embryos may lead to disturbing and far-reaching implications also became apparent, however. The lay participant according to whom any individual resulting from the (hypothetical) reproductive use of hELS would 'not be a real human being' for a lack of a unique DNA, for instance, showed how this perspective may have moral implications beyond the context of research. Clearly, it would be troubling to consider human clones (and indeed: identical twins) as having less moral status than other human beings. The second exercise probed the participants' views on the acceptability of research with hELS specifically by comparing hypothetical research scenarios with surplus embryos, research embryos, and hELS, respectively. These views were again noticeably in line with those taken in scholarly debates about the 'discarded-created' distinction **(Macklin 2000; Steinbock 2020; Devolder 2004, 2012, 2013; de Miguel-Beriain 2014)** and the '14- day rule' (**Cavaliere 2017; Hurlbut et al. 2017; Appleby & Bredenoord 2018; Williams & Johnson 2020; Peters 2021; Hyun et al. 2021; Nicolas et al. 2021)**.

Our participants' views suggest that these rules need not extend to hELS that lack the ('active') potential of human embryos— which incidentally may also have implications for non-viable human embryos **(Pereira Daoud et al. 2020)**. As previously discussed, if there is no real capacity to become persons, only considerations arising from the morality of interests can matter. Hence, only if these hELS were sentient, for example, would there thus be reason to restrict their research use. While most participants seemed to think of sentience as a hard research limit, it is not evident that this needs to be the case. As previously mentioned, actions that cause pain or discomfort can still be justifiable from the perspective of the morality of interests, albeit under conditions of proportionality and subsidiarity. This is not to say that hELS that lack potential are completely innocuous, however. Even though most participants indeed welcomed research with (non-viable) hELS as a morally preferable alternative to research with (viable) human embryos, a few of them still raised issues they believed warrant consideration. Even though most of these issues were not new, the fact that a beating heart was also mentioned as a morally relevant limit for research with hELS is an interesting finding due to a lack of theoretical grounding. In the ethical literature, a heartbeat is usually only considered to grant (symbolic) moral value if it represents 'a human individual in the process of becoming' **(Aach et al. 2017; Hurlbut et al. 2017)**. But the participants that mentioned this feature viewed it as a categorical limit for research with any hELS—even if they evidently lacked a potential toward human development. Our hypothesis is that these participants must have relied on a reversal of traditional approaches to moral status: instead of the feature (in this case, the heartbeat) deriving its moral significance from the value of the human being it denotes, the human organism (which, in this case, cannot become a human being) is taken to derive its value from that of its feature.

By contrast, if hELS were conceived to have the ('active') potential of human embryos, the question arose whether their research use too should be bound by the limitations of the Dutch Embryo Act. Of course, for participants that viewed this as implying that hELS would be persons with potential, any research that would treat them as mere instruments was deemed categorically wrong. But similarly to debates about human embryos, this was only a very small minority. For most participants, even if hELS were potential persons, they were not persons yet and could therefore be subject to research under considerations of proportionality and subsidiarity. Moreover, there may well be reasons to prefer their research use over that of human embryos even when both have an 'active' potential. Prominent reasons were, for example, that hELS do not require gametes, and therefore avoid the scarcity and burdens associated with oocyte donation when compared to research embryos; or that their 'artificiality' and the fact that they are specially created to serve as research material would make them more suitable for certain studies when compared to surplus embryos. For these participants, there were thus good reasons to regulate research with hELS differently from research

with human embryos. This led to discussions about the current Dutch ban on research embryos, which—if hELS had a potential akin to human embryos—would bar their creation, and the 14-day rule, the reasoning behind which could be easily evaded by the developmental plasticity of hELS.

Limitations and recommendations for further research

This study should of course be understood within the context of its limitations, one being the relatively small number of focus group and individual interviews it consists of, which prevents the generalization of these results to broader publics. Another potential limitation to note is that of selection bias in the pilot focus group and in the focus group and individual interviews with professional participants, all of which were selected by and from the networks of the research team. The same applies to the collection of the results, in which interviewer bias cannot be ruled out, and subsequent analysis, which necessarily involves a certain degree of interpretation and may therefore have been construed differently by different researchers. Finally, although participants were generally informed both prior to (in writing) and during (verbally) the interviews, it can also not be ruled out that certain misunderstandings of the science may have nonetheless remained.

Having that said, our study shows that the arguments participants articulated and the spectrum of positions they took with regard to the conceptual and moral qualification of hELS line up well with the arguments and positions found in the ethical literature. Even though the artificiality of hELS seemed to play a bigger role in lay group discussions, no other significant differences were found between professional and lay perspectives. Lay citizens thus seem quite capable of considering the development of hELS from an ethical perspective, which can hopefully help allay concerns about lay publics not being able to meaningfully participate in debates about the ethical ramifications of (novel) scientific developments. The fact that these perspectives also align well with several of the ISSCR's recently updated guidelines for research with hELS **(Lovell-Badge 2021; Lovell-Badge et al. 2021; ISSCR 2021)** further supports this thesis. The guidelines were unfortunately only updated after we had collected the data and could therefore not be taken on board during the interviews. But the participants' emphasis on tying research limits into particular ethical considerations, rather than into time in culture, maps nonetheless well onto the thrust of these recommendations **(ISSCR 2021)**. Another example can be found in the participants' greater preoccupation with 'viable' hELS, which corresponds with the ISSCR's advice to review research with so-called 'integrated' models—i.e., hELS that could come to have a developmental potential akin to human embryos—more stringently.

This is not to say that possessing 'a potential for further human development' was decisive in distinguishing between contentious from non-contentious research. Research with hELS that were conceived to be evidently incapable of developing

into human beings—called 'non-integrated' models by the ISSCR—also raised moral concerns in focus group discussions, for instance. These concerns were primarily linked to neural and brain development, which participants worried could make these structures sentient (albeit only in a very rudimentary sense). Whether and from whence this could be possible, as well as what that would imply for the acceptability of their research use, remains of course to be established. The alleged moral relevance of a heartbeat in entities that cannot grow into human beings is another issue that those involved in the development of guidelines for research with hELS may wish to further explore and connect with. But 'potential' clearly also need not provide a categorical moral basis for cutting-off research. Here, questions emerge about what we exactly mean when talking about 'potential' and what that does or does not imply for research with hELS that could come to possess it; an issue we—and hopefully others—will take up for further analysis.

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PART III

Discussion and Reflection

CHAPTER 6

Potentiality switches and the case for precaution: the Argument from Potential in times of human embryo-like structures

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ABSTRACT

Recent advancements in developmental biology enable the creation of embryo-like structures from human stem cells, which we refer to as human embryo-like structures (hELS). These structures provide promising tools to complement—and perhaps ultimately replace—the use of human embryos in clinical and fundamental research. But what if these hELS—when further improved—also have a claim to moral status? What would that imply for their research use? In this paper, we explore these questions in relation to the traditional answer as to why human embryos should be given greater protection than other (non-)human cells: the so-called Argument from Potential (AfP). According to the AfP, human embryos deserve special moral status because they have the unique potential to develop into persons. While some take the development of hELS to challenge the very foundations of the AfP, the ongoing debate suggests that its dismissal would be premature. Since the AfP is a spectrum of views with different moral implications, it does not need to imply that research with human embryos or hELS that (may) have 'active' potential should be completely off-limits. However, the problem with determining active potential in hELS is that this depends on development passing through 'potentiality switches' about the precise coordinates of which we are still in the dark. In our view, this provides a valid argument for precautionary measures, including the pre-emptive suppression of developmental potential beyond certain stages.

INTRODUCTION

The culture of blastoids **(Liu et al. 2021; Yu et al. 2021; Yanagida et al. 2021; Kagawa et al. 2021)** and gastruloids **(Morris et al. 2020)** from human pluripotent stem cells (PSCs) marks an important step forward in the refinement of—what we will refer to as—human embryo-like structures (hELS). It should be noted that there are many umbrella terms currently in use to describe these structures **(Rossant & Lam 2021)**, including 'stembryos' **(Veenvliet et al. 2021)** and 'stem cell-based embryo models' **(ISSCR 2021)**, but also that none of them is completely value neutral. While all seek to describe 3D clusters of PSCs that recapitulate aspects of early human embryogenesis *in vitro*, the fact that these clusters can differ significantly in terms of cellular origin (i.e., embryonic and/or induced PSCs), tissue composition (i.e., embryonic and/or extraembryonic membranes), and organizational complexity (e.g., pre- vs. post-implantation stages) makes them a visibly heterogeneous group. In addition to the increased experimental utility these structures provide, such as decoupled and bottom-up approaches to human embryology **(Posfai et al. 2021)**, refinement of hELS is driven by the hope it can strike a happy medium between opposite sides of the human embryo research debate: resembling human embryos closely enough to enable important avenues of research while steering sufficiently clear from them to avoid the moral discussions raised by their instrumental research use. Whether this hope is justified, is now prompting moral debate **(Denker 2006; Rivron et al. 2018; Sawai et al. 2020; Nicolas, Etoc & Brivanlou 2021)**. At the crux of this debate is the empirical question of whether or not (improved) human ELS (hELS) could come to achieve a developmental potential akin to 'natural' (or: 'fertilization-based') human embryos and the moral question of what that would imply for the acceptability of their use in research.

In this paper, we focus on the moral question and assume a positive answer to the empirical one for the sake of debate. That is, we argue from the particular and—presently—hypothetical scenario in which improved hELS would be able to develop until birth. In the first part of this paper, we revisit the so-called 'Argument from Potential' (AfP) in the traditional human embryo research debate. Here, we show that any attempt to justify (more or less restrictive) human embryo protective regulations with reference to the embryo's intrinsic moral status must require an appeal to the AfP, i.e., the argument that early human embryos deserve (some degree of) protection because they have the potential to develop into mature human beings. Moreover, we show that the AfP can best be understood in terms of a spectrum along which different versions are possible, each with different implications for the degree of protection it can confer. In the second part of this paper, we synthesize the findings of foregoing sections in order to illuminate difficulties with applying (versions of) the AfP that have so far received little scholarly attention. We first argue that, while the validity of the AfP has been criticized in light of the very developments leading to creation of hELS, these critiques remain inconclusive:

it is possible that the AfP can be maintained not only with regard to human embryo research, but also with regard to research with hELS. Next, we show that maintaining the AfP will nonetheless still require further characterization. At present, it is unclear how the argument should apply because, contrary to human embryos, it is much more challenging to identify whatever "switches" could be responsible for the inception of active potential in hELS. We conclude that this uncertainty might offer a valid reason for the present regulatory emphasis on precaution, for which pre-emptively building-in suppressing genes may offer a viable solution on most readings of the AfP.

THE AFP IN THE TRADITIONAL HUMAN EMBRYO RESEARCH DEBATE

With human embryos becoming available for research purposes due to developments in *in vitro* fertilization (IVF) and related biotechnologies at the end of the 20th century, came the question of whether and, if so, under what conditions, such research would be ethically and legally acceptable. This question stemmed from the widely shared intuition that human embryos were somehow morally special when compared to other (non-)human cells. Not because their scarcity made them valuable research material that should be used prudently, but because many viewed them as entities "toward which moral agents have, or can have, moral obligations" **(Warren 2011, 3)**: entities with (intrinsic) *moral status*, the research use of which, if acceptable, would require due diligence.

Accounting for the Special Moral Status of the Embryo

What could give human embryos a moral status that would restrain their instrumental use in scientific research? The fact that they are living entities would certainly not suffice to make this claim, as it would include far too much. A better candidate would be sentience: it is because sentient animals have needs and interests—including an interest not to experience pain or discomfort—that their instrumental research use is limited to important research aims only (proportionality), and specifically those that cannot be achieved through morally less sensitive means (subsidiarity) **(LaFollette 2011)**. What would sentience entail for human embryo research? The wish to steer clear from conducting research at developmental stages where human embryos might feel pain has certainly played a role in the **Warnock (1984)** Committee's recommendations leading to the influential 14-day rule, which prohibits the research use of human embryos beyond fourteen days post-fertilization and has since been adopted internationally **(Hyun, Wilkerson & Johnston 2016; Pera 2017)**. However, it also seems clear that, at least as far as sentience is concerned, the 14-day rule is overcautious. As it presupposes a degree of brain development that can only be acquired at fetal stages **(Lowery et**

al. 2007; Bellieni 2019; Derbyshire & Bockmann 2020), it seems fair to conclude that there is no reason to constrain human embryo research on this basis. Moreover, if sentience were all that counts and it does not stand in the way of using animals (or animal embryos) in research, it is unclear why its emergence would stand in the way of the similar use of human embryos.

Of course, it is precisely the fact that human embryos are human that many intuitively regard as making a moral difference. Could their humanity be a convincing ground for according them a (special) moral status that animals or embryos of other species lack? Most people would indeed agree that human beings have a higher moral status than other sentient non-human animals. This is reflected in the fact that, whereas sentient non-human animals might justifiably be used as research material under procedural and material conditions, similar use of human beings would be morally unacceptable and legally forbidden in all countries with human rights based research legislation. They may of course participate as *research subjects* based on their informed consent in studies approved by ethics review committees, but that is precisely what marks the difference with using them as *research material*. Why is it that human beings are thought to have this higher (or full) moral status that (as famously phrased by **Kant (1998)**) forbids their use as 'mere means'? In order to avoid charges of circularity and speciesism, we must have a reason beyond the mere fact that they are human.

It is here that the concept of *personhood* comes into view. Typical human beings are not just sentient animals; they are also *persons*. On John Locke's classical definition, a person is "a thinking intelligent being, that has reason and reflection, and can consider itself as itself, the same thinking thing, in different times and places" **(Locke 1997, 302)**. Persons are thus to be understood as beings with a capacity for rational self-consciousness over time, which provides them with a sense of personal identity as well as a capacity for (moral) agency **(DeGrazia 2005)**. While (most) sentient non-human animals do not meet these criteria and therefore enter the moral community only as moral objects, typical human beings are thought to also qualify as moral subjects. Despite the concept of personhood being able to explain why typical human beings are thought to have a higher moral status than (most) sentient non-human animals, it is difficult to see how it could apply to human embryos: whatever they are considered to be, they are clearly not persons in the Lockean sense. The claim that they might nonetheless deserve (some degree of) protection for their own sake (i.e., intrinsic moral status) would thus still require a further argument, for which the AfP is the only plausible candidate **(Stone 1987)**.

Full and Limited versions of the AfP

The intuitive appeal of the AfP lies in how it links the present embryo and later paradigmatic person or, in other words, in how it accounts for both the *continuity* and *discontinuity* between what the embryo currently is and what it has the potential to

become in the future. Nonetheless, advocates of the argument can and do differ with regard to which of these aspects they emphasize, and that in turn can lead to different views about the moral status of human embryos and the acceptability of their use in research **(Fig. 3)**. Those who emphasize the continuity aspect, generally take the AfP to entail that human embryos have the same ('full') moral status as human persons, which would imply that they should not be used as mere research material. An example of this stance can be found in the dissenting position to the Warnock Report by three of its Committee members, according to whom

> "… the embryo has a special status because of its development to a stage at which everyone would accord it the status of a human person. It is in our view wrong to create something with the potential for becoming a human person and then deliberately to destroy it" **(Warnock 1984, 90)**.

Another example can be found in the work of Reichlin, who argues even more explicitly, "human embryos must be treated as persons, since personhood is their destiny" **(Reichlin 1997, 7)**. We will refer to these interpretations of the argument as the 'Full Moral Status Versions of the AfP' or 'Full AfP', for short.

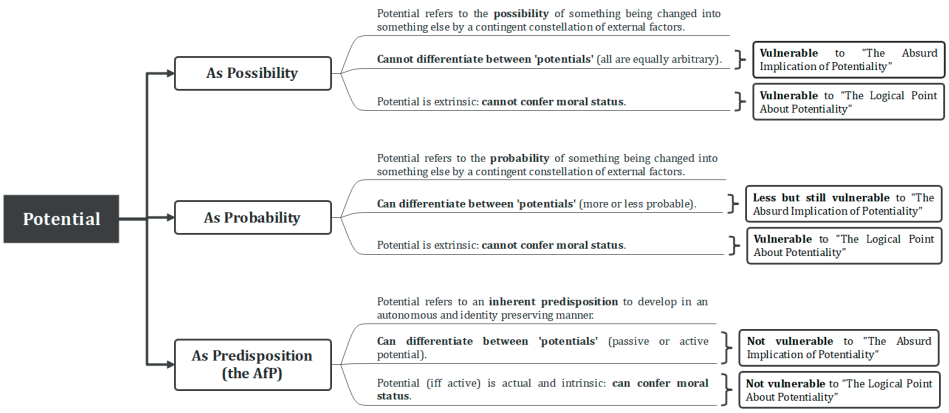

Figure 3: Chart of possible differences in AfP positions with regard to the moral bearing and onset of active potential.

By contrast, those who emphasize the discontinuity aspect, regard the fact that human embryos are only *potential* persons to mean that any moral status derived from this potential must be limited as compared to that of *actual* persons. This is often spelled out in 'gradualistic' terms, capturing the widely held intuition that the claim to consideration that human embryos (and human fetuses) have is initially weak, but that

its strength increases with later stages of development **(Poplawski & Gillett 1991; Kant 1998; Heinemann & Honnefelder 2002; Álvarez-Diaz 2007)**. Since this view is compatible with allowing human embryo research under conditions of proportionality and subsidiarity until later developmental stages **(Heinemann & Honnefelder 2002; Pugh 2014)**, it can typically be found in policy documents accompanying more or less liberal embryo research regulations. The Explanatory Memorandum to the Dutch Embryo Act, for example, states that "what makes an embryo worthy of protection … is its potential to grow into a human being" **(Tweede Kamer der Staten-Generaal 2001, 49)**, but it does not suggest that this protection should go beyond setting certain limits that still allow important research to proceed. As explained in an earlier report from the Dutch Health Council, the human embryo's moral "value is on the one hand determined by its potential to grow into an individual …, but on the other hand by the fact that this development has only just begun", thus making it conceivable that "other values and interests outweigh [its] worth" **(Gezondheidsraad 1986, 74-75)**. We will refer to these interpretations of the argument as the 'Limited Moral Status Versions of the AfP', or 'Limited AfP', for short.

Possibility, probability, and (active) potential

The AfP has also met with criticism. As conveyed by what Feinberg coined "the logical point about potentiality" **(Feinberg 1992, 51)**, nothing follows for the embryo's actual moral status from the fact that it might change into a future being whose moral status will then be uncontested. Moreover, as **Harris (1985)** and others **(Singer & Dawson 1988; Warnock 1987)** have argued, the argument would require us to protect much more than only human embryos:

> "To say that a fertilised egg is potentially a human being is just to say that if certain things happen to it (like implantation), and certain other things do not happen (like spontaneous abortion), it will eventually become a human being. But the same is also true of the unfertilised egg and the sperm." **(Harris 1985, 11)**

The subsequent and often reiterated 'absurd' implication that human gametes should then also be afforded moral status is meant to bring home that the AfP is better abandoned. These authors converge in that they interpret the concept of potential as the mere possibility of something being changed into something else by a contingent constellation of external factors (or, 'arbitrary things happening to it'). If that is what the AfP is taken to refer to, then it is indeed difficult to see how it could retain any moral bearing.

Other authors have argued that the embryo's potential should be understood in terms of probability, rather than mere possibility **(Noonan 1970; Engelhardt**

1986). Would this interpretation save the AfP from the foregoing criticisms? Perhaps it could take the brunt of the 'absurd implication'. If greater probability of maturing into a human being were to mean greater moral status, then it would be possible to draw moral distinctions based on the entity's (i) developmental stage (early vs. late), (ii) circumstantial environment (in vivo vs. in vitro), (iii) creation purposes (research vs. reproductive), and indeed (iv) organizational level (organisms vs. reproductive cells). However, it would still run up against Feinberg's logical point—i.e., the fallacy of deducing actual moral status from what the embryo is only in potential **(Feinberg 1992)**.

Advocates of the AfP, however, remain unconvinced by these criticisms, which they counter argue to be straw man fallacies **(Stone 1987)**. Contrary to what critics purport, the argument is not about personhood being an empirically possible (or probable) outcome, but rather about it being the outcome towards which the embryo is intrinsically predisposed **(DeGrazia 2012) (Fig. 4)**. As stated by Reichlin, the idea is that

> "… the embryo's development does not depend on external causes, rather on an inherent teleology that only demands certain environmental factors to be displayed: the embryo has itself the potential for full personhood, and does not receive it from outside." **(Reichlin 1997, 7)**

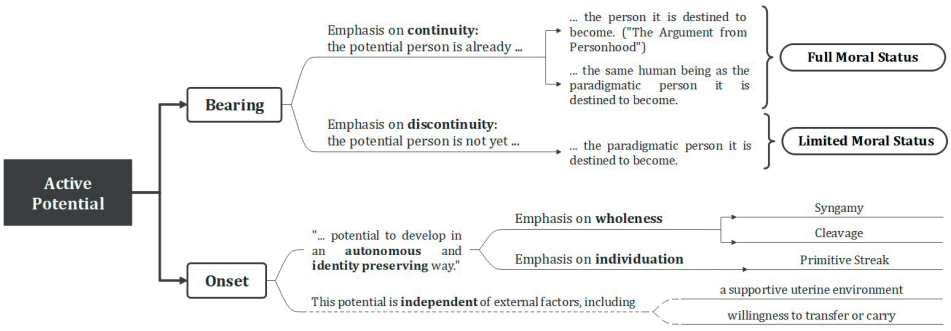

Figure 4: Chart of how potential could be interpreted differently and what these differences would imply for attributing moral status on that basis.

In the literature, this contrast in 'types of potential' is commonly explained in terms of Aristotle's distinction between *passive* and *active* potential **(Aristotle 1998)**, or Buckle's *potential to produce* and *potential to become* **(Buckle 1990)**. Whereas the former notion is used to refer to the possibility of an entity changing into something else by virtue of external causes, the latter is used to denote the *autonomous* and *identity*

preserving development that can establish a relationship between what the entity currently is and what it is intrinsically destined to become. On this understanding of the AfP, there is no logical gap between potential and actual moral status because the potential to become persons must already be an actual characteristic of the developing entity. As remarked by **DeGrazia (2012)**, this makes it a more cogent argument than its critics tend to suggest. Moreover, since gametes are separate organismal unities, they cannot be identical with the person(s) that arise from them. This means that the relation between gametes and future persons can only be one of producing, rather than becoming, and that the charge that the AfP would have the 'absurd implication' of also applying to the unfertilized egg and sperm therefore fails.

Different views on the moral bearing of active potential

Both Full and Limited versions of the argument allow for different ways in which they explain the moral bearing of active potential, depending (again) on whether they emphasize the continuity or the discontinuity in the relationship it establishes **(Fig. 4)**. Full versions can come in one of two variants. According to the version of the Full AfP that might just as well be referred to as the 'Argument from Personhood', the embryo is already the person it is destined to become in a more full-fledged sense. On this view, the later development of Lockean properties (such as self-consciousness, rationality, and moral agency) confirms, rather than establishes, the personhood that characterizes human beings at all stages of development **(Lee 2004)**. An alternative version of the Full AfP avoids equating personhood with species membership and departs from the notion that the embryo is the same human being as the paradigmatic person it is naturally destined to become. According to this view, human embryos have a strong interest in realizing their active potential, and that interest grounds a right to care and protection that is of equal strength to that of full-fledged persons **(Stone 1987)**. As observed by **Steinbock (2011)**, this reasoning is implicit in Marquis' 'Future-Like-Ours Argument' **(Marquis 1989)** because only beings destined to become persons could have valuable futures like ours.

Limited versions of the AfP also build on the notion of active potential as an actual and morally relevant characteristic of the developing entity, but without affording them full moral status on that basis **(Heinemann & Honnefelder 2002)**. One way of explaining this lower moral status has been suggested by DeGrazia. Given that, prior to the emergence of sentience, there can be no psychological unity that would link fetuses (or embryos) with the paradigmatic persons they will become at future stages of their lives, whatever interest they presently have in realizing their potential can only be weak **(DeGrazia 2012)**. While DeGrazia does not commit himself to the view that pre-sentient fetuses (or embryos) could have such an interest, the fact that he nevertheless presents it as a defensible position connects with his view that we are essentially animal organisms, rather than (embodied) minds. Clearly, this is also the position of those adhering to (Full

or Limited versions of) the AfP in the human embryo research debate **(Alvarez Manninen 2007)**. On the opposite metaphysical view, as defended by **McMahan (2002)**, the earliest possible stage at which a human being with active potential for paradigmatic personhood can be present is from 20 weeks of gestation onwards. Given that the fetus's time-relative interest in realizing this potential can only be weak (a point on which **DeGrazia (2012)** and **McMahan (2002)** converge), what follows is a version of the Limited AfP whose relevance is restricted to debates about the moral status of developed fetuses (and subsequent implications for late abortion and fetal interventions), not human embryos.

Different views on the onset of active potential

While advocates of the AfP thus agree that there can be no active potential prior to fertilization, there are different views as to the point from whence it can be acquired **(Fig. 4)**. Some see completion of fertilization as the obvious onset for active potential, as from syngamy onwards there is a living organism that is internally programmed "to develop in the species-specific way" **(Jochemsen et al. 2004, 88)**. The emphasis here is on autonomous development and organismic wholeness. Others doubt whether that can be enough to identify the pre-implantation embryo with the individual person(s) it may grow into. A first reason for this is that most cells of the pre-implantation embryo will contribute to the formation of extraembryonic tissues, rather than to the formation of the 'embryo proper', which only emerges with the process of gastrulation at around two weeks of development. A second and more important reason is the possibility for fusion or splitting occurring, which many take as evidence that embryonic development cannot be identity preserving prior to these stages **(Curran 1979; Buckle 1990; Persson 2003)**. The emphasis here is on individuation as a further condition for the onset of active potential.

For authors that maintain that active potential can only be ascribed to (post-) gastrulation embryos, the Full AfP could serve as an argument for underscoring the 14-day limit (and the development of the primitive streak, specifically) as a cut-off point for research, whereas the Limited AfP would only require imposing certain conditions on research beyond that stage. However, neither would provide convincing grounds for forbidding or regulating research with pre-gastrulation embryos. By contrast, authors who do not share this view on identity preservation could maintain that active potential (and the ensuing implications, which would again depend on whether they defend Full or Limited versions of the AfP) also applies to pre-gastrulating embryos. Reichlin, for example, maintains that, "the attainment of indivisibility … perfects the individuality of the embryo, but … does not show by itself that a new individual is present only at this stage" **(Reichlin 1997, 21)**.

THE AFP IN THE CURRENT HUMAN EMBRYO(-LIKE) RESEARCH DEBATE

In this paper, we assume that the ability to model embryonic morphology and functionality in clusters of human (induced) PSCs raises the prospect of further refinements resulting in hELS with a potential to develop into human beings. What would this scenario entail for their moral status and for how we should treat them in research? In terms of the foregoing discussion, it would seem that the crucial question is whether this potential should be qualified as passive or active. If passive, then it would be a matter of mere possibility and therefore not establish moral status. If active, then we would be dealing with cells that are at least morally equivalent to human embryos. For several authors, however, the developments prompting this very question only come to show that there can be no such thing as active potentiality in developmental biology, and that the cellular convertibility increasingly demonstrated by experiments in the field should be seen as the finishing blow to the AfP.

Ongoing debate about the validity of the AfP

Before hELS could be reported, Stier and Schoene-Seifert had already argued that the AfP could no longer be sustained in light of contemporary advancements in developmental biology **(Stier & Schoene-Seifert 2013)**. Their argument referred specifically to experiments with tetraploid complementation in mice, which presumably showed that a tetraploid environment could trigger induced PSCs (iPSCs) injected into aggregate embryos to convert back into a state of totipotency, ultimately generating offspring. Assuming the same would apply in humans, Stier and Schoene-Seifert argued that these insights represented a fatal variant of the absurd implication. After all, if adult cells can be induced back into a state of totipotency and ultimately develop into mature human beings, then this ability must be innate to all human cells and require only that the right environmental triggers are 'switched on'. Piotrowska has presented similar arguments **(Piotrowska 2020; Piotrowska 2021)**. In her view, the belief that human embryos develop autonomously into mature human beings is "an artifact of our pre-biotechnological past" **(Piotrowska 2020, 175)** that needs to be set aside. Keeping it would ignore the fundamental influence of an instructive (uterine) environment in the realization of the human embryo's potential to develop into a human being, rather than, for example, into a tumor.

The view that current science has no place for the notion of active potential has itself invited criticism, however. In his commentary to Stier and Schoene-Seifert, **Hyun (2013)** argued against stretching the implications of tetraploid complementation experiments in mice. Apart from the fact that these findings might not translate to human, they fail to provide evidence for the particular point Stier and Schoene-Seifert try to make. Rather than showing that active potential could be triggered in individual

iPSCs and that it therefore only needs to be released by appropriate external switches, the experiments seem to show that it can only emerge when clusters of cells interact as a unitary whole, which is not at odds with the notion of autonomous development. Although Hyun is explicitly not seeking to defend the AfP, which he interprets as necessarily and problematically implying that early human embryos would have full moral status on that basis (i.e., the Full AfP), his observations can be taken to support the AfP's central distinction between active and passive potential. A more categorical defense of this distinction in the current debate can be found in the work of **Denker (2015; 2021)**. Against the view that the signals for 'symmetry breaking'—which is crucial for germ layer development and body plan formation in mammalian embryonic development—come from the uterine environment, he argues that there is increasing evidence that these signals in fact emanate from the embryo itself **(Denker 2021)**. In other words, symmetry breaking is what the embryo autonomously does, rather than what happens to it. For Denker, these insights add credence to the view that "mammalian embryos are ... complete developmental systems, possessing active (not just passive) developmental potential" **(Denker 2021, 9)**. Moreover, he argues, the fact that this self-organizing capacity has also been reported in hELS suggests that these structures may also acquire the autonomous (or active) potential relevant for moral status. Which, ethically, he further suggests should be considered a "quantum leap with regard to the dignity to be ascribed to a colony of stem cells, moving it into the same ethical category as an embryo of that stage" **(Denker 2021, 9)**.

While the AfP remains empirically and analytically contested, this ongoing debate suggests that claiming its obsolescence in light of current insights in developmental biology might be premature. As it stands, the AfP may continue to play a role not only where the ethics and regulation of traditional human embryo research are concerned, but now also in that of research involving three-dimensional clusters of human stem cells (i.e., hELS). Clearly, active potential cannot be ascribed to hELS that model exclusively extraembryonic tissues (which should instead be referred to as extraembryonic *organoids*), but the argument might hold for structures that (also) model embryonic lineages.

Potentiality switches

If we suppose that certain hELS have active potential, this must mean that it is possible to control its emergence in a petri dish; i.e., that active potential can be 'switched on' by altering the composition of a cluster of cells that previously only had passive potential. This is not entirely new, as for those holding the view that the active potential of natural embryos starts at conception, the process of fertilization can also be seen as a controllable potentiality switch. For those who consider gastrulation to be (necessary for) the onset of active potential, allowing the embryo to undergo that process might also be considered such a switch.

In human embryos, it is clear where those switches are and what they imply for moral status (depending of course on whether one holds Full or Limited versions of the AfP). In hELS, however, it is far from clear how these switches can be identified: at what point in their development can hELS acquire active potential? This question is important because, as long as we are in the dark about the precise coordinates of the relevant switches, we cannot tell whether the material that researchers are working with may or may not be regarded as entities with (some degree of) moral status. In this connection, Denker sees an urgent need to elucidate under what

> "… conditions a group of stem cells may start the way to autonomy in the sense of gaining independence of pattern formation from outside signals, how this specific state of developmental autonomy can be detected, and how the process can be controlled." **(Denker 2021, 9)**

Here again, for active potential to be present, development must be both autonomous and identity preserving. Denker seems to regard this as one and the same potentiality switch that may change the moral status of hELS already at pre-gastrulation stages. As an advocate of the Full AfP, he therefore cautions that whatever the benefits for (clinical) science, experiments "leading to the formation of blastocyst-like or gastruloid constructs, should … for ethical reasons" **(Denker 2021, 9)** not be done with human (but rather non-human primate) stem cells. However, for those holding that an identity preserving development cannot begin at pre-gastrulation stages, the emergence of autonomy at those stages is at most a precondition for any later passive-to-active potentiality switch. Blastoids (like blastocysts) are not potential persons in their view.

An interesting question that illustrates the difficulties of applying the AfP in the current debate is whether hELS that contain the cells typically found in the embryo proper, but that are nonetheless incomplete in the sense of lacking (those that will form) extraembryonic ones, should therefore be regarded as lacking active potential. Gastruloids, for example, may be considered similar to the embryo proper in terms of how they form the derivatives of the three germ layers and undergo axis-formation *in vitro* **(Moris et al. 2020)** despite lacking extraembryonic tissues. **Sawai et al. (2020)**, who raise this question, qualify these structures as having only passive potential because of how they would require hypothetical scaffolding and culturing technologies in order to develop further. While these authors seem to conceive the use of these additional technologies as 'transformative' (i.e., in the sense of representing a switch from passive to active potential), it might also be conceived as merely supportive. Perhaps these technologies do not represent the change, but the 'help' that those structures would need in order to develop into mature human beings. How would this be qualitatively different from the help *in vitro* embryos need (i.e., transfer to an appropriate environment) in order to realize their active potential? As the active potential of *in*

vitro embryos does not depend on whether they are transferred to a womb, there might be a case for reasoning that a lack of extraembryonic membranes would similarly not prevent gastruloids from having active potential. Given that not all AfP advocates would consider identity preserving potential as something that can be present at stages where fission or fusion may still occur, this would imply that gastruloids could have a more secure claim to moral status than more complete hELS (e.g., human blastoids) and indeed human embryos (e.g., early human blastocysts) at pre-gastrulation stages.

The case for precaution

Talk of 'potentiality switches' remains of course hypothetical as long as we do not know whether any (improved) hELS would actually be able to develop into a mature human being. Noting that ethical concerns (including the ban on reproductive cloning) prevent us from doing the relevant experiments and that no offspring has so far been born from mammalian ELS, the question of how we should deal with this epistemological uncertainty remains in the meantime. At present, there seems to be a growing tendency to argue for precaution. Sawai and colleagues, for example, propose a pragmatic consistency approach in which research with hELS that have every component of natural human embryos (including extraembryonic tissues) should be regulated in the same way as research with natural human embryos at similar stages **(Sawai et al. 2020)**. This could practically imply restricting research with human blastoids as we would with human blastocysts (as currently done in Australia **(Australian Government 2021)**), even though it remains scientifically disputed whether these models in fact resemble human blastocysts in every respect **(Posfai et al. 2021)**. The scholarly consensus reflected in the Updated Guidelines of the International Society for Stem Cell Research **(ISSCR 2021)** is another example of similarly precautious conclusions. While presented as pragmatic, precautionary approaches such as these are arguably better defended on the basis that human embryos or equivalent entities deserve (some level of) protection for their own sake than on the view that they do not. Without a claim to moral status, there would be little to outweigh the price of refraining from important avenues of hELS research or for imposing regulative burdens on researchers other than a wish to avoid public concerns or sensitivities.

As a further precautionary measure, it has been suggested that one might think of genetically modifying hELS so as to suppress development beyond a certain point and to ensure that the entity could not possibly grow into a mature human being **(Rivron et al. 2018)**. This is sometimes referred to as building in 'suicide genes'. Of course, if the modification is built in at stages where active potential may already exist, this should be regarded as an active-to-passive potentiality switch, which as such would not serve to lessen AfP-based concerns. Building in such a switch would clearly be unacceptable on Full versions of the AfP. On Limited versions, it could be argued that meeting the subsidiarity requirement would be difficult, given that those genes could also have been

built-in at whatever stages clearly precede the possible emergence of active potential. But then indeed: what about a pre-emptive modification at those earlier stages?

One might think of doing this modification in the separate PSCs used to create hELS, thereby ensuring that the resulting clusters of cells do not develop beyond a certain point or that they fail to reach essential milestones. Whether this strategy avoids raising ethical concerns based on the AfP depends on whether it would prevent the emergence of active potential and ensuing moral status, however, and not all advocates of the Full AfP can be expected to agree that it would. Those adhering to the version of the AfP that we have earlier referred to as "the Argument from Personhood" might follow the reasoning of Doerflinger **(The President's Council on Bioethics 2004)**, the representative of the U.S. Catholic Bishops' Conference, in the earlier 'altered nuclear transfer' debate. This debate, which can be seen as a precedent to our discussion, centered on the idea of combining cloning and genetic modification in order to create non-viable human embryos as a supposedly morally neutral source of human embryonic stem cells for research and therapeutic purposes **(Hurlbut 2005)**. Doerflinger did not buy into this. According to him, if those non-viable human embryos would only differ from normal human embryos in terms of not being able to develop beyond a certain point, then the proposal would simply amount to creating human persons with a deliberately shortened life-span **(The President's Council on Bioethics 2004)**. Piotrowska refers to this precedent as a further illustration of the problems she sees with the AfP: apparently, its advocates would even have us protect entities without any potential to grow into a mature human being **(Piotrowska 2021)**. However, that seems too strong of a conclusion, as it ignores the specific reasoning underlying this version of the AfP and how it differs from all other AfP versions, namely that it sees active potential as a confirmation of personhood, rather than as a condition for attaining it. On all other accounts of the AfP, pre-emptively building in developmental roadblocks may well entail that there is no potential for personhood from the start and, therefore, no moral status. For this to hold true, however, it is essential that these roadblocks obstruct the potential for further development 'from within', rather than merely frustrating it 'from without'. Cutting short essential developmental pathways in (the precursor cells of) the embryo proper would seem to fulfill this requirement, but it is less clear whether the same would apply for cutting-off genes that only affect the capacity to implant. In light of the foregoing discussion on whether lack of extraembryonic tissues would stand in the way of ascribing active potential to gastruloids, one might similarly argue that blastoids in which the genes for implantation have been suppressed would ultimately be comparable to *in vitro* embryos to which transfer to a uterus has been denied. If the latter does not stand in the way of having active potential, then the same could perhaps also be said with regard to the former. Building-in suppressive genes that would instead affect the development of the embryo proper, might be regarded as a meaningful precautionary approach by most advocates of the AfP. Obviously, the need for such measures will be

more strongly felt by those adhering to (the remaining version of) the Full AfP, rather than to Limited versions. For the former, the case for building-in suppressing genes is a matter of allowing hELS (and human embryo) research in the first place. For the latter, it may still be important as a matter of avoiding the restrictions and regulative burdens of their use in research.

CONCLUDING REMARKS

If current embryo research regulations are to reflect the view that human embryos have intrinsic moral status and therefore deserve (some level of) protection, they can, as we have suggested, only be accounted for in terms of the AfP. Clearly, this cannot be versions of the AfP that ascribe full moral status to human embryos from conception, as that would require forbidding all forms of human embryo research.

In most countries, regulations permit research with human embryos, but only under conditions of proportionality and subsidiarity, and up to 14 days of development. With regard to the 14-day rule: were this to be accounted for in terms of the AfP, it could only be the Full AfP in its 'from gastrulation' version. However, this would undermine the case for an AfP-based justification of conditions imposed on research with pre-gastrulation human embryos. This tension does not arise on the widely held interpretation of the 14-day rule as a pragmatic rather than a principled line. Shifting that line to, for example, 28 days, or making it flexible as recently proposed by the **ISSCR (2021)**, is compatible with both versions of the Limited AfP.

While the AfP can thus be seen as underlying current embryo research regulations, it has always been contested. This criticism has gained further strength with developments in hELS-research, purportedly showing that there is no such thing as 'active potential'. If true, this would not only undermine the case for building regulations around the notion that hELS with significant developmental potential should be treated differently from those without, but also put current regulations of traditional embryo research on a much weaker footing than if the AfP would apply. This is important, because limiting important research in these fields is less easily justified if it cannot be done in terms of respecting the intrinsic moral status of human embryos and functionally equivalent hELS.

As this debate is ongoing, it seems premature to relegate 'active potential' to the dung heap of the history of philosophy. But if, as we have suggested for the sake of argument, the AfP can be maintained, it is not immediately obvious how it should be applied to the new field of hELS research. As we have suggested, the problem with determining active potential is that this depends on development passing through—what we have referred to as—'potentiality switches' about the precise coordinates of which we are still in the dark. In our view, this provides a valid argument for the present emphasis on precaution. A specifically interesting approach is to suppress developmental potential pre-emptively

beyond a certain point. While this would not be acceptable according to what we have called the 'from Personhood' reading of the Full AfP, it would seem a viable precautionary strategy on other versions of the AfP, notably when it involves obstructing developmental pathways that would affect the development of the embryo proper.

Interestingly, commentators who have argued that in times of hELS, it has become clear that the AfP can no longer be maintained, do not tend to conclude that it would thus also be impossible to ascribe moral status to human embryos or functionally equivalent hELS. For instance, **Piotrowska (2020; 2021)** and others **(Aach et al. 2017)** have proposed regulators do away with the whole idea that developmental potential might morally matter and instead consider simply which morally relevant features may arise in hELS. However, short of the actual capacity to feel pain (which is not realistic at embryonic stages), it is unclear what conditions we should think of (e.g.: primitive streak, heartbeat, neurological substrates, etc.) whose moral significance would not tacitly depend on the AfP.

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CHAPTER 7

General Discussion

GENERAL DISCUSSION

This dissertation inquired whether, and under what conditions, research with three-dimensional human embryo-like structures could provide an ethically acceptable alternative to the use of (fertilization-derived or 'natural') human embryos in research. The main objectives of this inquiry were (1) to advance the emerging debate on the ethics of human embryo models, and (2) to contribute to the development of ethically sound frameworks and mechanisms to govern their use in research. The first part of this chapter summarizes foregoing findings and conclusions; the second, synthesizes them for the purpose of answering the main research question and discusses the implications of that (provisional) answer in a broader research context.

SUMMARIES

Chapter 2

Chapter 2 aimed to provide a comprehensive overview of (human) embryo-like structures and to explore the ethical and policy issues likely to arise from their culture and research use for agenda setting purposes. The first part of the chapter went into our contemporary understanding of early embryonic development in mouse and human, followed by an overview of the approaches and limitations leading up to the generation of stem cell-based embryo-like models. Functional and morphological differences between existing pre- and post-implantation models were elucidated in detail (see **Fig. 1** and **Table IV** in Chapter 2), but the focus lay on 3-dimensional (3D) ones, which model early embryonic development more accurately. The following four models were the most important ones throughout this Chapter and dissertation: blastoids, ETS/X embryos, gastruloids, and Post-Amniotic Sac Embryoids (PASE). *Blastoids* model the blastocyst at peri-implantation stages and can differentiate into most embryonic and extraembryonic cell lineages. At the time, blastoids had only been cultured from mouse stem cells (induced and embryonic) and, even though they had been shown to induce decidualization in mice, development stagnated shortly after, which meant they could not be used for studying post-implantation stages. *ETS/X embryos* model gastrulation events at early post-implantation stages, during which the three germ layers from which the fetus would develop (namely, embryonic endoderm, mesoderm and ectoderm) form and begin to organize into an axis (i.e., symmetry breaking and primitive streak formation). ETS/X embryos had also only been cultured from mouse cells (although different combinations of embryonic, trophoblast and endoderm cells had been used), and shown to degenerate shortly after inducing decidualization in pseudopregnant mice. *Gastruloids* also model post-implantation events but up to later stages, including early phases of organogenesis (when the organs begin to develop). The gastruloids

reported at the time had only been cultured from mouse embryonic stem cells and could not generate neural cells nor extraembryonic tissues, which prevented them from being able to implant in a uterus. *PASE* were the only embryo-like structures that had been cultured from human (induced and embryonic) stem cells at the time. PASE model events associated with human amniotic sac development at post-implantation stages, but do not generate the extraembryonic tissues required for implantation.

The second part of the chapter reviewed the traditional human embryo research debate as a comparative normative framework and juxtaposed it against research with—at the time, mainly hypothetical—human embryo-like structures for ethical exploration. Our findings, which were intended to be explorative, mapped out challenges on conceptual, normative, and regulatory levels. On a conceptual level, we found that, even though fertilization and developmental potential are prominent criteria in contemporary embryo definitions, a universally accepted definition of 'natural' human embryos remains sorely lacking. This means that the question of whether (certain) human embryo-like structures qualify as human embryos can be answered in different ways. Since none of these models arises from the fusion of gametes, human embryo-like structures are likely to be *a priori* ruled out from qualifying as embryos in jurisdictions where fertilization is viewed as a necessary condition (e.g., Spain). Matters are less clear in jurisdictions where developmental potential lies at the essence of embryo definitions. If the emphasis lies on the potential to *initiate* organized embryonic development up to or beyond a certain stage—like the primitive streak, as is the case in Australia—the implication is likely to be that only a subset of embryo-like structures can qualify as natural embryos. Which subset that is will of course depend on the state-of-the-art.[3] Even more challenging are jurisdictions where the emphasis lies on the potential to *develop into a human being* (e.g., Belgium and the Netherlands), which presumably involves development up to or beyond birth. At present, human embryo-like structures are incapable of developing much further (let alone up to birth) for reasons yet to be understood, but given the fast-paced advancement of the field, it is conceivable that that understanding can soon be acquired. This means that, even though present-day human embryo-like structures do not qualify as human embryos in these jurisdictions, future and improved versions might. The challenge then is how to ascertain at what point these models have reached that stage. Laboratory experiments aimed at determining whether human models can develop up to birth would of course be ethically unacceptable.

3 In this chapter, the particular hypothesis was that PASE, gastruloid and ETS/X embryo-like structures might qualify as embryos under Australian law, whereas blastoids might not. This hypothesis stemmed from the fact that the blastoids cultured at the time did not seem to have "the potential to develop up to, or beyond, the stage at which the primitive streak appears" **(Research Involving Human Embryos Act, 2002)**, but that changed a year after publication with the culture of iBlastoids **(Liu et al. 2021)**. iBlastoids were more advanced than blastoid models previously reported, and eventually prompted the Australian National Health and Medical Research Council (NHMRC) to make "a decision based on the principles of statutory interpretation that iBlastoids come within the definition of a human embryo under the *Research Involving Human Embryos Act 2002*, and therefore require regulation and oversight" **(National Health and Medical Research Council 2021)**.

Can animal experimentation provide a solution? The literature suggests it can, as the historical fix to the similar epistemological challenge raised by Somatic Nuclear Transfer (SCNT) was indeed to infer from experiments with non-human mammals. Another historically suggested alternative was to resolve the epistemological challenge by evading it altogether. For example, by knocking out the requisite genes for viability, which, if done in human embryo-like structures, would preemptively rule them out from ever qualifying as human embryos. Altogether, our conceptual findings show not only how differences in embryo definitions have implications for the scope of research with embryo-like structures, but vice-versa, how research with embryo-like structures has implications for the scope of embryo definitions: whereas fertilization was already known to prove an inadequately narrow criterion, developmental potential may now also prove inadequately wide.

Of course, the question of whether human embryo-like structures conceptually qualify as human embryos should be distinguished from the moral question of whether and to what extent they deserve protection. For example, should human embryo-like structures possess features that many consider morally concerning, such as sentience, incipient brain activity, or an emerging human form, then normative protection may be warranted even if they clearly are not human embryos. The two questions coincide, however, when the criterion used to denote a cellular organism as an 'embryo' or 'non-embryo' is the same as the one used to discern between organisms with moral status from those without. We found that this is the case with developmental potential, for instance, which in the ethical literature is key in discerning between clusters of human cells that matter morally from those that do not. In the absence of this potential and short of other, intrinsically relevant features (e.g., sentience), human embryo-like structures (as well as natural human embryos) can at most have symbolic moral worth. If we suppose that human embryo-like structures can undergo prenatal development, however, matters become open to discussion. In the traditional human embryo research debate, it was common to understand this potential in one of two ways: 'passive' or 'active'. When understood as an extrinsic ability to produce a certain outcome given the right external cues, the potential was 'passive' and could at most confer moral value by association. When understood as an intrinsic ability to undergo changes to itself and, therefore, as 'active', it could confer either full or (as more commonly held) gradually increasing moral status. Which of the two, if any, would apply in the hypothetical scenario of viable human embryo-like structures is now prompting scholarly debate. Although this remains a contested position, some have argued that the concept of 'active' potential cannot be maintained in the face of stem cell-based models of embryos precisely because these models show that cellular potential is nothing more than contingent possibility (i.e., all potentials are passive) and, therefore, unsubstantiated grounds for moral status. But even if we suppose that the concept can be maintained, the chances are that it would still not apply to all embryo-like structures alike. For those that consider numerical

identity a necessary condition for 'active' potential, for instance, the concept can only bear moral relevance once the developing entity has become indivisible (or, in other words, at stages after which organismal fusion and fission can no longer occur). More particularly, this means that only the human organisms at stages past the development of the 'embryo proper', after which fusion and twinning is no longer possible, can have (a certain degree of) moral status on this basis, whereas human embryo-like structures (and natural human embryos) preceding these stages cannot.

From this, a few challenges stood out for policy. On the one hand, if (certain) human embryo-like structures do not conceptually qualify as human embryos, it follows that their research use will be subject only to the rules imposed on research with human cells and tissues more generally, which could also imply prioritizing their research use over the use of animals and natural human embryos from a subsidiarity perspective. Given that human embryo-like structures may raise moral concern even when they do not conceptually qualify as human embryos, there may be a regulatory lacuna here. On the other hand, if (certain) human embryo-like structures do qualify as human embryos, the question becomes how contemporary regulatory restrictions (should) apply to them. One straightforward implication is that research with these models is likely to be prohibited in jurisdictions where research with natural embryos is either forbidden or categorically constrained to the use of surplus ones. A further issue is how to account for research with human embryo-like structures in jurisdictions that forbid human cloning for research purposes. A less straightforward issue, and one that is currently prompting much debate, is how to apply current time limits (such as the 14-day rule) to entities whose development is not synchronous with that of natural human embryos of the same age. In jurisdictions where the rule is tied to particular developmental features (such as the United Kingdom, which explicitly mentions the primitive streak), this issue can be avoided, but in jurisdictions where it is not (such as the Netherlands), it is difficult to see how the rule could be applied meaningfully to human embryo-like structures. Restrictions regarding human embryo modeling will thus need to be tied to morally relevant features, rather than only time in culture. This, in turn, requires we (re)consider the material grounds for regarding certain developmental features (such as the primitive streak) as categorical cut-off points for research; a task that is only likely to be further complicated by the increasing ability to bypass and 'on-and-off' switch certain features in embryo-like models.

The chapter concluded with an urgent call for (re)consideration of the three main issues arising from ethical exploration. In particular, the need (i) to reconsider current embryo definitions, (ii) to reevaluate 'active' potentiality as grounds for moral status, and (iii) to rethink the 14-day rule and moral weight of (non-)viability in regulation.

Chapter 3

Chapter 3 was prompted by the first publications to report the successful generation of

so-called human blastoids, i.e., 3D embryo-like structures created from human (induced) pluripotent stem cells that model the blastocyst stage in early embryogenesis. The aim of Chapter 3 was to use these advancements as a case study that could illustrate and expand upon the agenda-setting issues set forth in the previous chapter. Since only few and decoupled embryo-like structures had been cultured from human cells at the time of writing Chapter 2, the issues we had identified as requiring further thought remained largely hypothetical. The successful culture of 'integrated' human embryo-like structures provided a first workable and real-life framework to probe these issues.

There are many advantages to the generation of embryo-like structures, especially when cultured from human cells. They provide unprecedented bottom-up approaches to early developmental biology, overcome the species-specific differences of animal studies, and are less burdensome to create in large (or small) quantities as well as more amenable to laboratory modification than natural human embryos. In addition to these technical benefits, human embryo modeling is also increasingly encouraged by the belief that it can provide a normative 'win-win' policy: tapping into the benefits of research with natural human embryos by modeling them ever more closely, while at the same time circumventing the ethical sensitives and legal restrictions of traditional human embryo research by remaining sufficiently unlike them.

This 'win-win' policy of course assumes that human embryo-like structures are and will remain just that: faulty or incomplete models of embryos, rather than actual ones. For 'non-integrated' human embryo-like structures that are lacking in important ways (as is the case in the human gastruloids reported in 2020) this is not really an issue. Morphological and functional differences, such as a lack of relevant cell types and limited developmental potential, set them visibly apart from natural human embryos while still enabling promising avenues of applied and fundamental research. Indeed, many research questions require simplification, and many human embryo-like models are scientifically useful exactly because they do not model the organismal complexity of natural human embryos in every respect.

'Integrated' human blastoids are presently also far from perfect human embryo replicas, and improving their fidelity will likely require parallel human embryo research, which should serve as a sobering note when counting on short-term benefits of the aspired policy. Nonetheless, their first-ever culture marked an important step forward in precisely that direction. Despite remaining technical limitations, these models were able to differentiate into all the cell types from which the fetus and supporting tissues would normally develop. Once remaining limitations are overcome, the question becomes how to ethically and legally distinguish between human embryo-like structures that are (still) just models and those that should more appropriately be regarded as human embryos. The paradox that emerges here is that the more similar these models become to natural human embryos, the less useful they might be in reducing or replacing natural human embryos in research. There is a tipping point

between similarity and identity; a point after which research with human embryo-like structures collapses into research with human embryos. Reaching that tipping point may not be ethically problematic in itself, but it will (need to) bring back the ethical and legal monitoring human embryo-like models were meant to circumvent. Since it is very difficult to determine where precisely this tipping point lies, it may be prudent to err on the side of caution and avoid creating perfect replicas in order to keep the envisioned policy a 'win-win'.

The lack of consensus on how to define human embryos, as discussed in Chapter 2, muddies the waters further. For scientists, it confounds the limits of research with particular types of (improved) human embryo-like structures, which may differ significantly even between neighboring countries. For politicians, it opens possibilities to have it both ways: benefitting from research with however perfected human embryo-like structures while taking the moral high ground with regard to natural human embryos. Should (animal) embryo-like models turn out to be viable, for example, then jurisdictions where the emphasis lies on fertilization could follow Spain's precedent in the SCNT-debate and maintain that these models cannot be human embryos per definition. Programmed non-viability could enable similar strategies in jurisdictions where the emphasis lies instead on developmental potential, such as the Netherlands. Since the resulting entities would not be human embryos under Dutch law, the Netherlands could allow their creation and research use without having to lift the moratorium on the creation of human embryos for research purposes. Of course, politically loaded definitions such as these would be at the least problematic when used to avoid, rather than address, the ethical and legal questions raised by novel advancements in human embryo modeling.

One of these ethical questions is what to think of the so-called 'potentiality argument', and of 'active' potential in particular. The generation of human blastoids from (induced) pluripotent stem cells underscores the debate reviewed in Chapter 2, in which some scholars have argued that the ability to manipulate cellular differentiation *in vitro* demonstrates that developmental potential is entirely a matter of contingent factors that can be switched on-and-off arbitrarily. Should they be correct, then human embryo research could no longer be restricted based on the claim that (only) early human embryos have the 'active' potential to develop autonomously, and could therefore be allowed to proceed even beyond fourteen days. Should their conclusion be premature and the concept of 'active' potential withstand, then two side-notes are still worth making. The first one is that 'active' potential is perfectly compatible with the view that human embryos have an initially low—albeit gradually increasing—moral status and can, therefore, be used in research under conditions of proportionality and subsidiarity. In other words, subscribing to 'active' potential does not have to translate to granting full moral status on that basis. The second one is that 'active' potential presupposes numerical identity between the embryo and the later person(s) that would develop

from it and, therefore, as many have argued, that the first possible stage during which it can be established convincingly is after twinning and fusion can no longer occur. This means that the concept of 'active' potentiality can only gain moral traction at post-implantation stages and, therefore, that both early blastocysts and blastoids cannot qualify for moral protection on that basis.

Other ethical issues are likely to arise precisely in relation to structures that are clearly not human embryos, and whose research use therefore falls outside the scope of human embryo regulatory frameworks. If these human embryo-like structures are used to research stages beyond the development of the primitive streak, which is prohibited in human embryos by the 14-day rule, then certain developmental features, such as beating hearts and incipient brains, may be especially sensitive for society. But even in the hypothetical scenario that these features could develop up to a point of sentience, it is unclear why that should be regarded as a categorical cut-off point for research. While in natural human embryos these features could be seen as markers of the mature human beings they are growing into (and, therefore, grant a certain degree of moral value), no such argument is available for human embryo-like structures that are clearly incapable of growing into human beings.

Chapter 4

Chapter 4 reported on empirical findings pertaining to the participants' confidence in (the regulation of) research with human embryo-like structures. Theme 1 focused on how participants intuitively felt about the topic, and underscored a spectrum of perspectives: as expected in relation to emerging biotechnologies, participants ranged between positive, negative, and ambivalent outlooks on human embryo modeling. Those with a positive outlook emphasized the benefits of human embryo modeling and their confidence in our societal ability to monitor and control the development of the field; those with a negative outlook expressed skepticism about its utility, motivation, and monitoring. In between were participants whose views of scientific research as morally indeterminate, and/or (lack of) knowledge about comparable emerging biotechnologies, evoked ambivalence. The fact that participants ranged between these perspectives regardless of expertise, with both lay and professional participants swinging between ends of the spectrum, suggests that scientific knowledge does not necessarily promote positive attitudes toward specific avenues of research. Still, professionals were visibly less prone to cynicism than lay participants were, which we hypothesize to relate to what the literature describes as 'deference to scientific authority' or, in other words, the professionals' greater familiarity with the governance mechanisms that underpin scientific research.

Theme 2 focused on what would make participants feel (more) confident about the field, and suggested that disparities in confidence could be overcome if human embryo modeling were regulated based on (at least) the following three criteria. The

first criterion was to regulate *the domain of application*: human embryo-like structures became especially contentious when conceived as material for commercial and reproductive applications. (Whereas commercial applications raised concern because of how they could lead to the commodification and profit-driven use of human material, reproductive applications raised concern predominantly because of how they were conceived in combination with cloning and eugenic practices). The second criterion was to regulate *the development of morally concerning features* in human embryo models. The question is whether the particular features our participants suggested (developmental potential, and specific features in organogenesis, such as a heart(beat) or a central nervous system) can justifiably be considered as such (as indicated later in this summary, and to which we return in more detail in Chapter 5). The last criterion was to regulate *collaborative design through public engagement* for normative, instrumental, and substantive reasons, as coined by Stirling.

Reflection on the findings of Theme 1 and 2 as a whole suggests that human embryo modeling raises concern on both a *general* and *specific* level. Concerns of the general kind arose especially in view of human embryo modeling being perceived as another step toward potentially deplorable dominion over (human) life. Principled judgments about the relationship between humans and the world were especially common in lay groups, and bore greater resemblance to the commonly found 'Playing God' framework in intersecting fields of research. In the focus group with professionals, the concern seemed to stem rather from a precautionary stance: an attempt to avoid hubris in cases where it may no longer be clear whether the benefits of research outweigh its burdens.

Concerns of the specific kind arose especially in view of the application of the technology. When applied to *non-reproductive* research contexts, for instance, human embryo modeling was welcomed as long as the models in question did not possess the features participants considered morally concerning. Examples were a heart(beat), a central nervous system, and developmental potential but, as previously mentioned, it remains to be established if and how much these features matter morally. Whereas a central nervous system is fundamentally linked to the morally relevant capacity of experiencing pain, it is unclear how a heart(beat) could be morally relevant in and of itself. Only if the heart(beat) were conceived to indicate the ongoing development of a new human being could it have moral meaning, but then still, it would not be the heart(beat), but rather the underpinning capacity to develop into a human being, that would bear actual moral relevance. While the moral bearing of developmental potential remains disputed in the scholarly literature, it is noteworthy that the participants had similar discussions to those found in the literature: whereas some took it to matter morally but not enough to halt research, others viewed it as a categorical limit. These results seem to back the normative distinction between 'integrated' and 'non-integrated' models that the International Society of Stem Cell Research (ISSCR) had suggested concurrently to our data analysis, which is based on the view that greater

integration may result in developmental potential. Yet they also underscore the lack of consensus on what potentiality can mean morally, and, therefore, that developmental potential alone says little about what conditions to impose and what limits to draw in research with entities that possess it.

The *reproductive* application of human embryo modeling was another major (albeit unforeseen) point of concern arising from the discussions. In the design of our empirical study, we had consciously limited our inquiry to non-reproductive contexts in an effort to avoid adding complexity to the discussions needlessly. This turned out to be an unproductive strategy, as participants were visibly concerned about—and consensually argued against—the possibility of creating offspring from human embryo-like structures. Even though the reasons to condemn the reproductive application of human models so strongly were not spelled out, the participants' associations to cloning and eugenics seem to indicate the specific concern of the technology one day being used to create modified clones on unprecedentedly large scales. While we cannot establish with certainty whether this is what participants had in mind when combining concepts of cloning and eugenics, it certainly is a new dimension that would benefit from additional reflection.

When understood within the context of its methodological limitations, the empirical findings discussed in relation to Theme 1 and 2 provide interesting avenues for further experimental research and theoretical analysis. This is especially the case with regard to the reproductive application of human embryo modeling, and the moral and logical validity of the features of moral concern our participants suggested. The Chapter concluded therefore with a call to fellow researchers in the humanities and social sciences to validate these findings and improve upon them.

Chapter 5

Chapter 5 discussed findings related to how the participants denoted and defined human embryo-like structures (Theme 3), followed by findings related to the considerations upon which they would afford human models moral protection, if any (Theme 4). In Theme 3, consensus was found in the view that the words we use matter, and in particular, that we should pick the words we use based on whether they reflect the (dis)similarity between natural human embryos and human embryo-like structures effectively. The problem was coming to an agreement on what these terms should be (whichever ones were used, were perceived to have inadequate normative connotations), and on which criteria they should be based (i.e., fertilization, and/or a potential to develop into a human being). Notably, questions of the latter kind arose not only in relation to human embryo-like structures, but also in relation to natural human embryos. These findings echo the scholarly emphasis on improving terminology for the many embryo models currently available, as well as the agenda-setting input of Part I with regard to the indeterminacy of traditional criteria in human embryo definitions

and the urgency to reconsider them.

In Theme 4, it became clear that the protection participants afforded to human embryo-like structures depended on whether or not these models were perceived to possess the features they considered morally relevant. Among the more prominent features were those associated with sentience and a potential for continuous human development, both of which are commonly found in the ethical literature. The only feature we found that seems to lack theoretical grounding is a beating human heart, which some considered to provide a cut-off point for research with human embryo-like structures regardless of their developmental potential. As a whole, however, the findings discussed in Theme 4 align well with the arguments and positions typically found in ethical debates about how moral status—including what that would imply for 'the 14-day rule' and 'the created/discarded distinction'—and reasons beyond that can ground normative protection. The contrast we found in the degrees of moral status different participants afforded to early forms of human life (including human embryo-like structures) maps especially well onto the distinction McMahan draws between the Morality of Interests (MoI) and the Morality of Respect (MoR), for instance. The relatively low protection many afforded to early forms of human life seemed to stem exclusively from the view that organisms at these stages cannot yet meet the necessary threshold to qualify as entities 'on equal moral footing with ourselves' (i.e., persons). On these views, the only kind of considerations that could matter for moral status are therefore those that fall within the MoI (e.g., sentience), which in the ethical literature is taken to mean that the protection afforded on this basis could permit research under conditions of proportionality and subsidiarity even beyond embryonic stages. The high or even full moral protection other participants afforded to early forms of human life seemed to be based instead on two versions of the 'argument from potential': one according to which organisms with potential are due gradually increasing moral status, and one according to which they are due full moral status. Whichever version one holds, of course extends only to human embryo-like structures with similar potential. In the ethical literature, this potential is only thought to matter intrinsically if it is 'active' or, in other words, inherently and autonomously driven. Interestingly, the 'artificiality' of human embryo-like structures was taken by some to mean that whatever potential these models have—for example, a potential to actually produce offspring—would still be qualitatively different from the potential of natural human embryos. In other words, some perceived the 'artificiality' of human embryo-like structures as *a priori* ruling out their potential from ever qualifying as 'active'. Which may of course lead to the troubling implication of deeming human clones (and indeed: identical twins) as having less moral status than other human beings.

What do these views suggest for the regulation of research with human embryo-like structures? Should the rules imposed on human embryo research apply to them? When these models were conceived to lack a developmental potential akin to natural human

embryos, the answer was predominantly negative, which may incidentally also have implications for research with non-viable human embryos. But this does not mean that their research use was considered completely innocuous. Should human embryo-like structures have interests, for instance, then there may be good reasons to protect them based on the MoI. The question is just to what degree: while most participants seemed to think of sentience as a categorical cut-off point for research, this is not evident from the perspective of the MoI. A feature that also seemed to cause contention (even) in models conceived to lack developmental potential was a beating human heart. As previously mentioned, it is unclear on what grounds this feature could derive moral relevance in the absence of developmental potential, which is why it may be worthwhile for regulators to further explore and connect with this issue. By contrast, if human embryo-like structures were conceived to have the developmental potential of natural human embryos, the answer was 'it depends'. It depends, for example, on whether that potential is understood as 'active' (or 'passive'), and on whether 'active' potential would make them 'potential persons' or 'persons with potential'. In our discussions, most participants held the former view, and considered research with human embryo-like structures acceptable under conditions of proportionality and subsidiarity. Moreover, even if the potential and ensuing moral status of human embryo-like structures were to be conceived as equal to that of natural human embryos, there may still be reasons to prefer research with the former to the latter. Prominent reasons were that human embryo-like structures bypass the challenges associated with oocyte donation, or that their 'artificiality' and special creation for research would make them more suitable material for certain research purposes than (surplus) natural embryos.

Taken together, Theme 3 and 4 show that lay citizens are quite capable of considering the ethical ramifications of research with human embryo-like structures. The fact that the aforementioned findings also align well with the thrust of the International Society for Stem Cell Research's (ISSCR) recommendations (particularly the emphasis on tying research limits into ethical considerations rather than time in culture, and on distinguishing between models that may manifest a developmental potential akin to natural human embryos from those that do not) supports this thesis. These findings are of course reassuring, but they also raise further questions. Questions arise specifically when it comes to what is meant by 'potential', and what that does or does not imply for research with human embryo-like structures that could come to possess it; an issue we take up in Chapter 6.

Chapter 6

Chapter 6 aimed to explore what, if any, implications the so-called Argument from Potential (AfP) could have for the regulation of research with human embryo-like structures. In order to focus on this moral question, we departed from the theoretical scenario in which future and improved human embryo models could come to achieve

the potential to mature into actual human beings.

We set the stage for discussion by first reviewing the rationale and moral bearing of the argument in its traditional context. The AfP essentially holds that human embryos have greater moral value than other (non-)human cells because only they have the unique potential to develop into persons. Historically, the argument has been taken to provide one of the very few grounds available to afford human embryos moral status, especially at early stages. The fact that it can account for both the difference and the continuity between what the embryo is in its present form and what it has the potential to become in the future, appeals to the intuitions of many, but different implications follow depending on which of these aspects is emphasized. Those that emphasize the continuity aspect, take the AfP to ground human embryos a 'full'—or at least, high enough—moral status that would prohibit their use in research as 'mere means'. We refer to this version as the Full version of the AfP or Full AfP, for short. Those that emphasize the difference, take it to ground a moral status that is initially only low, but which increases as the embryo progresses throughout pre-natal development. Unlike the Full AfP, this version of the argument is not only compatible with allowing research under conditions of proportionality and subsidiarity, but also with permitting it up to later stages. We refer to this view as the Limited version of the AfP or Limited AfP, for short.

The AfP has not been without criticism. One of these critiques is what Feinberg termed 'the logical point about potentiality', or the gap between deriving actual moral status from potential future states. A second one is known as 'the absurd implication' of the AfP, according to which consistent application of the argument would entail affording equal moral status to gametes. What these critics have in common, is that they seem to interpret the AfP as referring to the possibility of something being transformed into something else by a constellation of external 'things happening to it', in which case it is indeed difficult to see how it could retain any moral bearing. Others have proposed that the argument should be understood as referring to probability, rather than possibility, but it is questionable whether this approach would provide a more viable solution to the foregoing critiques. Since this view would allow drawing distinctions based on (i) developmental stage (early vs. late), (ii) circumstantial environment (*in vivo* vs. *in vitro*), (iii) creation purposes (research vs. reproductive), and indeed (iv) organizational level (organisms vs. reproductive cells), it perhaps could take the brunt of 'the absurd implication'. But it would still run up against Feinberg's 'logical point', unless of course the 'moral status' to be conferred on this basis were derivative from the actual persons for whom the realization of that potential matters. In that case, however, the argument would no longer confer moral status in the *intrinsic* sense of the word, and would therefore not provide sufficient grounds for categorically restricting (or prohibiting) research on this basis.

For advocates of the AfP, both criticisms remain unconvincing because they

mischaracterize the argument. Whereas the potential critics have in mind refers to the possibility (or probability) of X passively undergoing change by virtue of external causes ('passive' potential), the type of potential the argument is actually supposed to refer to is the one in which X actively contributes to the change it undergoes ('active' potential). Since 'active' potential requires in particular that the relation between present and future X is autonomous and identity preserving, there is no logical problem or absurd implication involved. However, there are prominent differences with regard to the point at which advocates of the AfP consider this potential to be identity preserving. Some see fertilization as the obvious onset; others doubt whether the criterion can be met at stages preceding gastrulation. Reasons for the second view are that, at stages foregoing gastrulation, most cells will form extraembryonic tissues (rather than the embryonic layers that will form the human embryo's body), and fusion and fission can still occur, meaning that embryos at these stages cannot yet be numerically identical with the individuals that emerge from them. On this account of identity preservation, only post-gastrulation embryos can be ascribed moral status based on the AfP, which could serve as a reason to underscore the 14-day rule as cut-off point for research on Full versions of the argument, or to permit research beyond fourteen days under condition on Limited accounts. For those that maintain that the identity criterion can be met at stages prior to gastrulation, however, the implications of Full and Limited accounts would of course also apply to embryos at earlier stages.

The second part of the Chapter aimed to contextualize the (different) views of the argument in relation to research with human embryo-like structures specifically. We began by briefly reviewing how the cellular convertibility demonstrated in embryo models is resurging debate about the feasibility of drawing distinctions in moral status based on 'active' (or 'autonomous') and 'passive' (or 'non-autonomous') potentials. Even though this debate is far from settled, its very existence suggests that to dismiss the AfP in light of current insights in developmental biology would be premature. The particular conclusion that the AfP may hold not only in research with natural human embryos, but now also in research with (improved) human embryo-like structures, does not have to be discouraging for either field, however. The first crucial question is how the developmental potential in question would (or should) qualify. Whereas active potential can ground an intrinsic claim to moral status (however strong, depending on Full or Limited readings), passive cannot. Since most human embryo-like structures model only partial aspects of embryogenesis, this means that the AfP is likely to not even arise in many cases. In cases where it does, it is still important to bear in mind that only Full versions of the argument would necessarily imply categorically cutting-off research on this basis, while Limited versions could allow it under conditions of proportionality and subsidiarity. At what point these implications apply, further depends on underlying views about the stage in which development can justifiably be conceived as identity preserving, which those for whom numerical identity matters take to mean that active

potential and associated moral status can only be acquired at post-gastrulation stages.

When regarded in relation to the hypothetical scenario in which human embryo models could arguably manifest a developmental potential akin to natural human embryos, the AfP also raises a series of new questions that can benefit from additional reflection. The first one is whether erring on the side of caution is worth its price. Though it may sound easy on the ears, taking a pragmatic consistency approach in the absence of evidence that (non-human) embryo-like structures could result in offspring will come at the cost of promising medical knowledge. Is this an acceptable shift in the balance research seeks to strike between the reasons to protect early (forms of) human life and the reasons to use them? A second issue is how to deal with the possibility of engineering active from passive potential—and vice versa. Has (active) potential become an attribute that can be switched 'on' and 'off' arbitrarily? What then is the threshold between conducive switches (that 'provide help') and transformative ones (that 'provide change')? These questions arise of course most pressingly in relation to human embryo-like structures, but advancements in CRISPR-Cas9 could make them equally conceivable in relation to natural human embryos. For example, if scientists succeed in building-in so-called 'suicide genes' pre-emptively. Could that possibility prevent the AfP from arising? If so, would it matter if, when or where suicide genes are built? Human gastruloids provide an interesting illustrative framework for these issues, as they contain the cells and model the stages associated with active potential in the traditional debate, while lacking extraembryonic tissues.

DISCUSSION

Embryo-like structures are three-dimensional clusters of pluripotent stem cells developed to model (aspects of) early embryogenesis *in vitro*, but differences in terms of cellular origin (e.g., iPSCs and/or EPSCs), tissue composition (e.g., embryonic and/or extra-embryonic), and organizational complexity (e.g., pre- vs. post-implantation) makes them a visibly heterogeneous group of models. While some of them are clearly different from embryos, others already seem able to recapitulate embryonic development to the point of scientists conceiving improved versions as virtually indistinguishable from them. The most recent examples of this are the mouse models two groups of scientists reported in August 2022, which were shown to be able of undergoing development *ex utero* until stages typically found at nearly half the gestation time in mice **(Amadei et al. 2021; Veenvliet et al. 2021)**. In one of these studies, so-called 'sEmbryos' were transferred to an artificial womb **(Veenvliet et al. 2021)**, thereby also offering the first proof-of-concept that embryo modeling and ectogenesis techniques could be used concurrently.

While present-day human models do not recapitulate embryogenesis either as closely or as far as contemporary mouse models do, they are a similarly heterogeneous group.

Some human models recapitulate limited segments of embryonic and extraembryonic tissue development, like the PASE reported in 2017, which model the development of the human amniotic sac. Others, only limited segments of embryonic development, such as the human gastruloids reported in 2020, which "form derivatives of the three germ layers organized spatiotemporally, without additional extra-embryonic tissues" **(Moris et al. 2020, 410)**. Yet others recapitulate both more thoroughly, such as the human blastoids reported in 2021, which included the formation of both embryonic and extraembryonic membranes and seem therefore capable of modeling human development more fully and continually. These differences are ethically important, especially considering the recent breakthroughs reported in mice. Should similar improvement in humans result in structures that are morphologically and functionally equivalent to natural human embryos, could their culture and research use be ethically permissible? Vice versa, should they steer sufficiently clear from natural human embryos, does that make their culture and research use morally innocuous? In short, can research with—present and future—human embryo models be ethically acceptable and, if so, under what conditions?

Provisional Answers: An Explorative Roadmap and Tentative Route

While I am not in the position to give a conclusive answer to this question, the aim of this dissertation has been to provide an explorative roadmap to reach a provisional one and—based on how I think we should travel that roadmap—the provisional answer would be yes. Research with human embryo-like structures can be ethically acceptable, even if some models become virtually indistinguishable from natural human embryos. The more pertinent question is *to what extent* it can be acceptable, and I argue that that will depend on what kind of ethical reasons we would have to restrict the use of specific human embryo-like structures in research. Let me explain what I mean by following the analogy of an exploratory roadmap along which there are up to three possible crossroads **(Fig. 5)**; each with successively narrower 'exits', or implications for research **(Table VI)**.

Table VI: Exit number and ensuing implications for research.

N º	Includes	Implies
1	Structures that evoke exclusively extrinsically motivated reasons to restrict research (i.e., structures that lack moral status; e.g., strictly extraembryonic structures).	Procedural restrictions (e.g., informed consent; comparable framework: research with human tissue)
2	Structures that evoke extrinsically *and* intrinsically motivated reasons to restrict research (i.e., structures with moral status) but which are not persons (yet) (i.e., structures without full moral status).	Procedural *and* material conditions (e.g., ethics review; comparable framework: animal research)
3	Structures that evoke extrinsically *and* intrinsically motivated reasons to restrict research (i.e., structures with moral status) and which are (or may become) persons (i.e., structures with full moral status).	Categorical restrictions or prohibitions (e.g., 14-day rule; comparable framework: human embryo research)

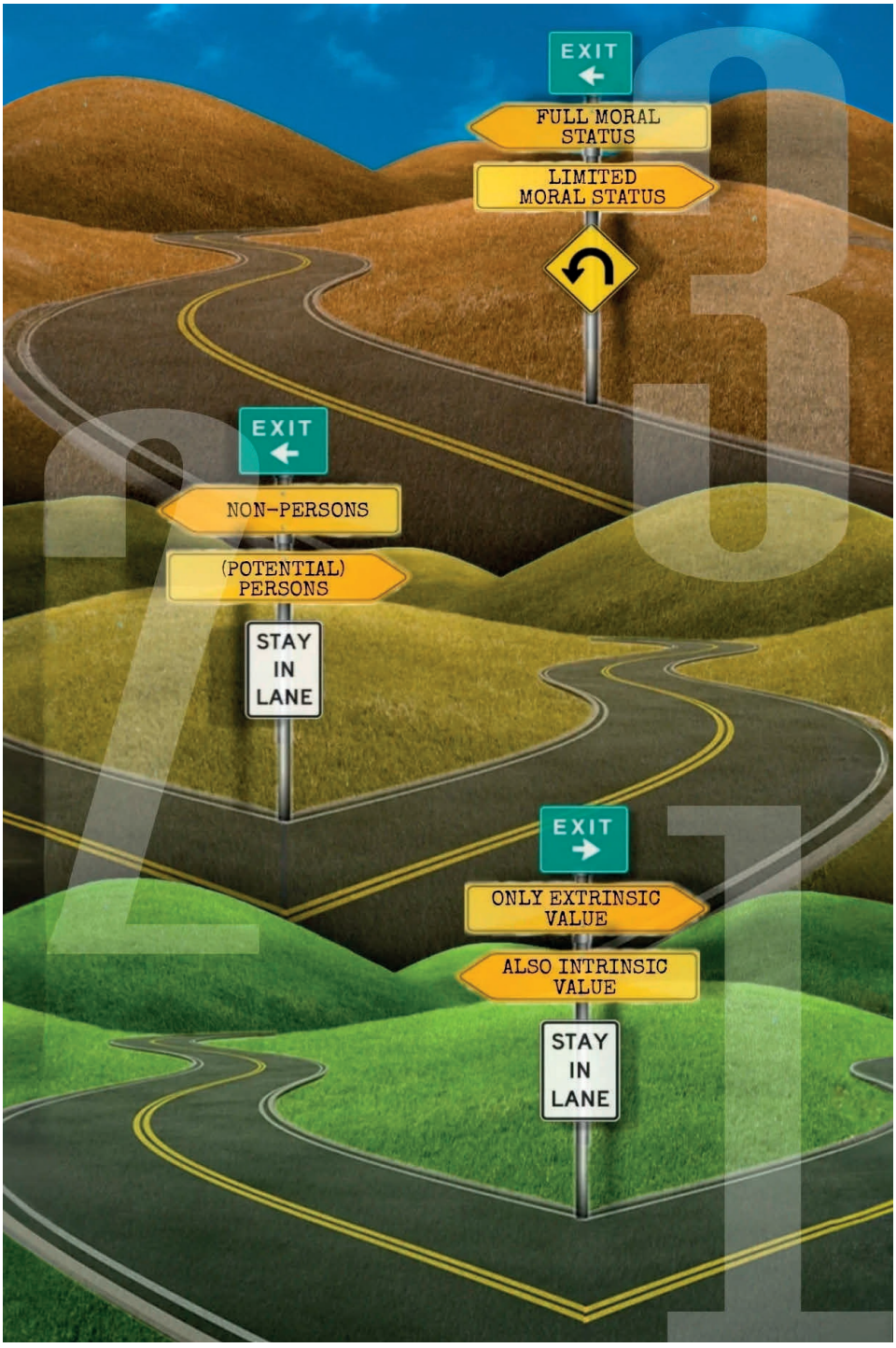

Figure 5: A representation of the three crossroads and respective exits.

The First Crossroad: Just Cells or Beings with Moral Status?

The heterogeneity of human embryo-like structures means that different models might raise different ethical reasons in favor or against their use in research. But the findings described in this dissertation show that these ethical reasons can also differ in kind. Whereas some reasons could compel us to restrain research with certain human embryo models by reference to the value they have for beings with moral status (i.e., extrinsically motivated reasons), other reasons could compel us to do so by reference to the value they have in themselves (i.e., intrinsically motivated reasons). Understanding this difference is important because whenever reasons of the latter kind apply the scope of acceptable research narrows down significantly. Intrinsically motivated reasons refer to qualities that are of non-derivative or inherent value. They are what ethicists commonly refer to as the constituents of moral status and, as put by a lay participant in our focus group interviews, the reason "we would not need to … have a discussion about bricks. Because bricks are bricks, right?" Since intrinsically motivated reasons ground moral status, they have priority over extrinsically motivated ones and set limits to the degree to which the latter can matter in the moral balance. The first crossroad in the roadmap is therefore asking ourselves what ethical reasons could stand in the way of the culture and research use of specific models, and in particular, whether these reasons would be only extrinsically or also intrinsically motivated.

Should the only reasons we have to permit or restrict research with particular models be extrinsically motivated ones (i.e., be exclusively derivative from how that research affects—human and non-human—beings with moral status), then those models would be no different from mere cells and the scope of permissible research would be similar to what is currently permitted in research with human tissue more generally. Let us call this **Exit 1**. Exit 1 is limited to structures that evoke only extrinsically motivated reasons. These reasons are inevitable and they can provide strong policy grounds. It is for example for good reason that the commercial application of research with human cells is only allowed with the informed consent of cell donors. Such motives can be conceived in relation to human embryo models and were explicitly mentioned by lay and professional participants in our focus group interviews. Exit 1 does not make it impossible to differentiate between types of human cells, however. A possible interpretation of extrinsically motivated reasons is that some can confer *symbolic* moral value, that is, value something would have by virtue of being an emblem of something else **(Strong 1997)**. Zygotes may differ from other single cells in terms of what they conventionally stand for (i.e., the beginning of human life), and that might give us reason to grant them greater protection in research. For example, because disrespecting that symbol would adversely affect beings with moral status in some way **(Strong 1997)**. If we suppose that some embryo-like structures as well as some natural embryos (namely those that lack active potential, as I argue in later sections) would end up at this exit, it might thus still be possible to distinguish between them. However, since beings with

moral status can be affected differently and how they are affected can change over time, policies based on extrinsically motivated reasons must remain amenable. This means that the scope of permissible research with models at Exit 1 could be more ample and open to concession than research with models at later exits.

At the time of writing, it seems that research with most types of human embryo-like structures would probably end up at Exit 1. In my view, this means we might have convincing reasons to impose certain procedural conditions on the research use of these models, but also that, insofar as these reasons are exclusively extrinsically motivated, they cannot provide strong enough grounds to justify prohibitions. The issue is what to think of the increasingly conceivable prospect of present-day models improving to the point of becoming entities that could matter in their own right (i.e., entities with moral status). Should that be the case, then there would not only be additional reasons that compel us to restrain their use research, but also weightier ones. In order to determine how weighty these reasons would be, however, we would need to travel onto the next crossroad.

The Second Crossroad: Non-Persons or (Potential) Persons?

The second crossroad would be reserved for research with entities that possess qualities of non-derivative value or, in other words, entities with moral status. At present, it seems unlikely that human embryo models possess such qualities, but the point is that research could be acceptable under conditions even with models that do. One of the main threads of this dissertation has been that the philosophical concept of personhood can ground a difference in 'domains of moral status', and that difference is most noticeable in how we treat human versus non-human animals. While both clearly have interests, the instrumentalizing (sometimes destructive) use of non-human animals in important avenues of research is ethically justifiable to many while nobody in their right mind would attempt to justify a similar use of human beings (and sometimes non-human primates). To denote this difference, I will refer to the domain that includes entities with moral status but that are *not persons* (i.e., moral patients) as the route leading up to **Exit 2**, and the domain that includes entities with moral status that are *persons* (i.e., moral agents) as the one leading up to **Exit 3**. What would each exit imply for human embryo modeling?

Exit 2 in human embryo modeling could theoretically justify restricting certain research avenues with human embryo models that can experience pain (for example, cosmetic applications), but it is hard to see how it could provide a hard research limit for all applications considering that research can be done with sentient animals. Moreover, in typical human development, sentience can only be acquired at much later (fetal) stages and we would thus no longer be strictly speaking about restrictions on embryo models. Should there nonetheless be reason to think that some human embryo-like structures possess the (rudimentary) substrates required to experience pain, then

those models could end up at Exit 2. In that case, research with such models might not only be ethically acceptable under conditions of proportionality and subsidiarity, but sometimes also ethically preferable—to, for example, research with sentient animals—under distinctive proportionality conditions.

Unless Exit 3 can somehow apply to human embryo-like structures, the road would end there and we would have to conclude that these models could not raise the kind of intrinsically motivated reasons that could justify prohibiting their research use. But how could this Exit 3 even apply? For the sake of a concise answer, I assume it is unambiguous to state that human embryo models are clearly not persons—at least, not in the sense of possessing the capacities traditionally associated with personhood, such as rational thinking, moral agency and self-reflection—and that it can therefore be difficult for non-ethicists to imagine how the moral status of persons could (indirectly) still apply to them. Yet these readers should be reminded that, even though natural human embryos have never been persons in that sense either, their *potential to become* persons is sometimes used as an argument to ground prohibitions that effectively compel us to treat them as if they are persons already (at least beyond fourteen days). As discussed in Chapter 6, it is possible that a similar reasoning could apply to human embryo models that have become virtually indistinguishable from natural human embryos. This means that a positive answer to the question of whether particular human embryo models (could come to) have the potential to become persons would be the only possible way for human embryo modeling to end up at Exit 3.

The word 'become' in the previous sentence is used judiciously, as it is meant to refer to *active* potential specifically. In the human embryo research debate, active potential was thought to necessarily involve autonomous and identity-preserving development, and therefore to preclude entities such as gametes (which on these grounds can produce but never become persons, i.e., only have passive potential) from moral consideration. In my view, an immediate implication of the traditional distinction between active and passive potential is that human embryo-like structures that model strictly extraembryonic membranes can at most have the latter. In these models, there is no developing organism that could preserve its identity throughout development (i.e., they are in a way no different from organoids) and therefore no convincing basis to speak of an active potential for personhood. Since it is furthermore unlikely that these extraembryonic 'organoids' could have (other) intrinsically relevant qualities, it seems that neither Exit 2 nor Exit 3 would be available to them. The only convincing ethical reasons we could have to restrict the use of these particular models in research would thus have to be extrinsically motivated ones, which would amount to no more than accounting for the procedural conditions associated with Exit 1.

Research with human embryo-like structures that model the development of embryonic membranes, regardless of whether they do so in combination with extraembryonic ones, could be a different story, however. Should these structures be potential persons (in the

sense of being capable of undergoing autonomous and identity-preserving potential), then some might argue that their research use should end up at Exit 3. The findings of this dissertation cast doubt on this approach because it remains disputable whether active potential can ground the moral status that would warrant categorical restrictions on research. (For that, active potential would need to ground the *full* moral status of actual persons but it is unclear how potential persons could derive the moral status of that which they effectively are not (yet)). In fact, it might even be disputable whether active potential by itself can be significant enough to trump the moral status of sentient animals structurally. I will return to this point at the next and last crossroad, but for now, let us depart from the more conservative position according to which active potential offers sufficient reason to prohibit research with the models that possess it (Exit 3). Which (if any) human embryo models could these be?

As we have seen in the course of this dissertation, the concept of active potential is still open to interpretation. At the crux of it, is the question of from what point can development be considered identity preserving and in particular, whether or not it requires numerical identity. Those for whom numerical identity does not matter (cf. **Reichlin 1997**) could maintain that potential persons begin at stages preceding primitive streak formation, but only if they did so in combination with the view that potential persons have *full* moral status would that need to imply prohibiting research at these stages. The way I understand it, this combination of premises would be the only way possible to maintain that the instrumental research use of human entities with active potential (which would effectively make them similar to natural embryos) should be completely off-limits. But as far as I can tell, this is not a commonly held view in the ethical literature and it would still leave room for research with models that only have passive potential (such as research with what I referred to as extraembryonic 'organoids'). By contrast, those for whom numerical identity matters (cf. **DeGrazia 2005**) are unlikely to maintain that human embryo models can have active potential at stages preceding primitive streak formation. Having only passive potential at these stages, research with human embryo models would likely end up at Exit 1, which in practice means there would be no intrinsically motivated reasons to restrain research with human blastoids even if they were virtually indistinguishable from natural human embryos (which present-day versions still are not). They could at most have symbolic moral value. At stages after primitive streak formation, research with human embryo models with active potential would end up at Exit 3 for those that grant full moral status on that basis and presumably at Exit 2 for those that do not. The first view could imply prohibiting research with models such as gastruloids (when improved), whereas the second could allow it under conditions of proportionality and subsidiarity.

The Third Crossroad: Full or Limited Moral Status?

At this point in the roadmap, it is again possible to take one of two exists. Which of

them should we take? The first exit is comparable to the stance reflected in current human embryo research frameworks, which effectively treat potential persons as if they already had the full moral status of actual persons by typically ruling out their research use after primitive streak formation (Exit 3). However, this exit does not seem to be coherent with how we would intuitively afford moral status (e.g., Chapter 5), nor is it consistent with universal principles of logic (e.g., **Feinberg (1992)**) and law (e.g., human reproductive rights). Prominent ethical background theories about personhood **(Locke 1694; Harris 1985; Warren 1997; Goodman 1998; Kant 1998; DeGrazia 2005)**, including **Aristotle's (1998)**, to which the concept of active potential is often attributed, also ultimately prioritize actualities over potentialities and, therefore, cannot support the view that potential persons should have the full moral status of actual persons either. By contrast, the second exit (and the one I would advise we take), does not require adjusting fundamental intuitions, principles or theories in any significant way in order to reach a reflective equilibrium. On this account, our moral belief system is already coherent and mutually supportive. Assuming that this set of beliefs is as widely shared as this dissertation suggests, one would additionally expect Exit 2 to come closer to the reflective equilibrium most readers would reach. In sum, while both exits might be defensible on more limited coherence justification methods in ethics, I would argue that only Exit 2—according to which active potential could ground some albeit not full moral status—would actually cohere with the wider set of beliefs we already fundamentally agree on.

Implications and Further Issues: Mind the Gaps

Exit 2, however, does not cohere with the prohibitions currently associated with the use of human embryos in research. For example, while there might be some (extrinsically motivated) reasons that justify drawing distinctions between the research uses of supernumerary and specially created human embryos on this account, these reasons are not of the kind that could justify banning their special creation, let alone justify structurally giving precedence to the use of animals in research. Exit 2 could also include (intrinsically motivated) reasons that could justify distinguishing between pre- and post-primitive streak formation stages. However, these reasons would again not be weighty enough to ground the hard research limits (such as the 14-day rule) commonly enforced by contemporary human embryo research laws. From a coherence point of view, taking my suggested route (or, a "U-turn to Exit 2" **(Fig. 5)**) would thus still imply adjusting some of the judgments currently enforced by human embryo research law. In particular, lifting prohibitions and subjecting research to ethical review. This implication would of course not follow for those that remain unconvinced by my preferred route. In that case, it may be possible to maintain that the rules of Exit 3 should continue to apply to research with human embryos. But that must also mean extending these rules to research with human embryo-like structures that have become

virtually indistinguishable from them, which, in countries that prohibit research cloning and the special creation of human embryos, would effectively mean banning research with some human embryo models. This may seem too obvious to mention, but given the past and present record of cherry picking in embryo research policy **(Alkorta, Berian & Rodríguez-Arias 2013; Dondorp & de Wert 2017)**, it certainly is no luxury.

Whichever the eventual route, the roadmap outlined in this dissertation renders ethical considerations more tractable and provides a better grip on those that currently are or can be challenged. Therefore, it can function as a preliminary tool for the scientists and policymakers wanting to navigate the moral landscape of research with human embryo-like structures, and as a starting point for humanities and social scientists to join the landscaping. To contribute to that end, I would like to conclude by agenda setting what I think are the most pressing questions and implications of this roadmap for current practice, and by highlighting issues outside the roadmap that might be worthwhile investigating further.

For scientists and policymakers, the question that I think requires further thought most urgently is whether the grounds currently used to draw normative distinctions between types of human embryo-like structures reflect the qualities that matter morally. At the time of writing, the scholarly consensus expressed in *inter alia* the Updated Guidelines of the **ISSCR (2021)**, and which is starting to be formalized by law in some jurisdictions (cf. **Dondorp et al. 2021; Starza-Allen 2022; Dondorp & de Wert 2022**), is that distinctions between models should be based on their degree of cellular integration. The roadmap (and route) outlined in this dissertation endorses the justifiability of drawing moral distinctions between different types of human embryo-like structures, but it raises the question of whether (non-)integration can provide a morally sound denominator. The reason why (non-)integration matters on these views is that it denotes the structures that "could potentially achieve the complexity by which they might realistically undergo further integrated development" **(ISSCR 2021, 64)** from those that cannot, which is reminiscent of the Argument from Potential. Yet the subsequent emphasis on "if cultured for additional time in appropriate conditions or, theoretically, if transferred to a uterus" **(ISSCR 2021, 64)** suggests that that potential is interpreted as mere possibility, which means it could not provide the kind of intrinsic reasons that could preclude research, i.e., it could not ground moral status. As discussed in Chapter 6, this is not what advocates of the argument traditionally had in mind. It could of course be an intentional choice to discard that traditional interpretation, but in view of current knowledge, the problem with that strategy is that it might be premature. On the other hand, if we consider (non-)integration from the perspective of the traditional Argument from Potential, the concept might also ultimately run into problems. Here, the issue is that understanding non-integration as the mere absence of extraembryonic tissues (as is currently the case) might still not reflect the issues that matter morally. For example, because it would preclude research with blastoids

but not with gastruloids, while the opposite should be true if only structures at post-primitive streak formation stages can have active potential. In either case, the problem of upholding (non-)integration as a normative divisor in policy and research is thus that it risks being fallible, which might come at the cost of (striking) a morally just balance.

Is 'active potential' a better alternative? That is an important question for the humanities and social sciences. Although active potential seems to be the only (intrinsically motivated) reason available to limit research with organisms at early embryonic stages, the concept remains empirically and analytically contested. The fact that active potential might now also have become something that can be engineered at will—in human embryo-like structures and, with gene-editing technologies, in the future perhaps in human embryos—adds fuel to the fire as it makes the concept even less tangible. Should increased manipulability be a convincing reason to discredit the moral bearing of active potential altogether? While I would argue that the concept could continue to support moral distinctions between organisms at pre- and post-primitive streak stages, I must admit that it is unclear to me whether and how these distinctions should translate to practice. After all, where should the line between 'needing help' (conducive) and 'needing change' (transformative) lie? Should research with organisms that lack active potential be free from ethical review, or is the concept so slippery that there is still something to be said about making it reportable? To address these questions meaningfully, we will need to do more than trying to make the ethics of human embryo modeling fit traditional human embryo research frameworks. Redirecting our efforts towards questioning whether some of those frameworks might in fact require revisiting in light of contemporary advancements is, I think, the best place to start.

Finally, at the borders of this explorative roadmap are issues pertaining to the artificiality and reproductive application of human embryo-like structures. These issues fall outside the scope of my inquiry, but nonetheless played a notable role during the interviews I held in the context of this research study. The artificiality of human embryo models was notable because of the contrast we found in how different participants valued it (Chapter 5). While it is unclear to me how artificiality could diminish the intrinsic value of the research material (as some participants seemed to suggest), it might be worthwhile to deepen what the concept should mean for its extrinsic value and the proportionality and subsidiarity of its use in research. Another issue that could benefit from further consideration is the theoretical possibility of using human embryo models for reproduction. In the design of our empirical study, we had wrongly assumed that it would be unnecessary to discuss the reproductive application of human embryo-like structures because of how far-off (if not altogether far-fetched) it was considered at the time. This topic eventually turned out to be a major point of contention during focus groups, however. Why is it that scholars do not seem interested in considering the reproductive use of these models? Is it because such applications are considered scientifically impossible, or rather because they seem to lack clinical

utility and compelling justification? The Updated Guidelines of the ISSCR seem to conceive of that possibility, and in particular to suggest that, while it might become possible to create models that could be used reproductively, that should not be allowed because it "lacks scientific rationale or is ethically concerning" **(ISSCR 2021, 9)**. This prompts the further question of what to think of using human embryo-like structures for reproduction should there ever be compelling justification. What arguments might rise in such a scenario? While safety concerns could provide obvious reasons to restrain from such applications, some of the participants we spoke to seemed to have other reasons in mind to object to it (often in relation to issues at the interface of cloning and eugenics). It would be worthwhile to validate and expand upon these issues in order to develop adequate policies proactively.

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ADDENDUM

PUBLIC SUMMARY

Scientists are now able to bring together various types of pluripotent stem cells to cultivate cell structures that resemble human embryos at certain stages of early development. These so-called 'human embryo-like structures' could offer an (according to some, ethically and legally neutral) alternative to the use of human embryos in research. For example, because they can be produced on a large scale without requiring invasive procedures (egg donation) and because they are not subject to the laws and regulations that govern research with human embryos. In addition, the use of these structures allows certain elements to be added or removed, which enables unprecedented bottom-up approaches to the study of early human development. The goal of the research described in this book was therefore to determine whether and, if so, under what conditions scientific research with human embryo-like structures can indeed provide an ethically acceptable alternative to the scientific use of human embryos.

The first part of this research focused on exploring the various types of (human) embryo-like structures and the potential conceptual, ethical, and legal issues that their use in scientific research could raise. In this part of the research, it was found that even though most embryo-like structures are still cultured from animal stem cells, several human variants have also already been created (such as 'blastoids', 'gastruloids', and 'Post-Implantation Amniotic Sac Embryoids' (PASE)). Each denotes a group of cells whose organization and differentiation resemble those of a human embryo at a certain stage of early development. 'PASE' recapitulate several events related to the development of the amniotic sac. 'Gastruloids' resemble the cells of the 'embryo proper' at the gastrulation stage (which begins with the formation of the primitive streak at around two weeks of development) and lack the cells that would produce extra-embryonic tissues (such as the placenta) and which are necessary for implantation and further development in the uterus. 'Blastoids' resemble embryos at the blastocyst stage (around 5 days of development) and consist of all the cell types typically necessary for further development: those that would produce the 'embryo proper' and those that would produce extra-embryonic tissues. For the modeling of even earlier embryonic stages, there are (currently) no corresponding embryo-like structures, although research with recently discovered 'extended pluripotent' stem cells may change this. The so-called 'ETS/X-embryos', which also consist of embryonic and extra-embryonic tissues, have not yet been cultivated from human stem cells but appear to be capable of modeling early development from the blastocyst stage to early organogenesis (around days 5.5 to 8.5) in mice. At the time of writing, all structures are imperfect and have limited developmental potential, but scientists around the world are working to their further improvement. That further improvement makes it conceivable that research with what begins as human blastoids could one day also be used to replicate and study the development of human embryos at later stages. Even though not all research questions require a recapitulation

of the entirety of cells typically found in early human development, it seems likely that this will become increasingly possible in the future. This inevitably leads to the question of how to distinguish between structures that are still no more than models and those that are such perfect replicas that they have essentially become stem cell-derived human embryos. The paradox that emerges here is that the better human embryo-like structures become at modeling early human development, the more difficult it will be to maintain that they provide an ethically and legally neutral alternative to research with human embryos. Where that transition precisely lies is not easy to answer: while in animal research, the birth of healthy (and fertile) offspring would provide the ultimate proof-of-concept, ethical reasons prevent us from doing these experiments with embryo-like structures cultured from human stem cells.

This part resulted in the identification of questions for further research on conceptual, moral, and policy levels. Since there is no universally accepted definition of human embryos, different answers are possible to the conceptual question of whether (certain) human embryo-like structures can or cannot be considered human embryos. Since none of these structures arise from the fusion of gametes, it is unlikely that they can be considered embryos in countries where fertilization is deemed a necessary condition of human embryo definitions (as is the case in Spain). Whether they should be considered embryos in countries where the emphasis of embryo definitions lies on developmental potential (i.e., the ability to undergo continuous development), is less clear. If the emphasis lies on the capacity to initiate early human development (as is the case in Australia), only a subset of human embryo-like structures will likely be considered embryos. Which subset that is will depend on the state-of-the-art. But if the emphasis lies on the potential to develop into a human being (as is the case in Belgium and the Netherlands, and which presumably at the least implies development until birth), it becomes even more challenging to identify which structures can and cannot be considered as such: as mentioned earlier, it is simply not possible to test whether or not human embryo-like structures have that potential in ethically acceptable ways. This leads to an epistemological challenge. The conceptual question of whether human embryo-like structures do or do not qualify as human embryos should however be distinguished from the moral question of whether and to what extent they deserve protection. If (certain) human embryo-like structures possess characteristics that can be considered morally relevant (such as early brain activity, the ability to feel pain, or the potential to become persons), then a certain level of protection may be warranted regardless of whether they qualify as human embryos. For example, if human embryo-like structures have the same potential to become persons as human embryos, this must mean that they have the same moral status (and are therefore due the same degree of protection) as the human embryos that are protected on that basis. The question remains, however, on what basis that potential can have moral meaning; a question that is further elaborated on in later chapters. From a policy perspective, these findings pose specific challenges. On the one hand, if we assume that (certain) human

embryo-like structures are not to be considered embryos (for example, because they do not have the potential to become human persons), their scientific use will only have to be subject to the (less strict) rules that apply to research with human cells and human tissues in general. From a subsidiarity perspective, this could also mean that the use of these human embryo-like structures should take precedence over the use of animals and human embryos in research. Since it is conceivable that human embryo-like structures might elicit moral sensibilities regardless of whether or not they are embryos, however, there may be a protective gap here. On the other hand, if we assume that (improved) human embryo-like structures can be considered embryos, the question arises as to whether and how the restrictions that apply to human embryo research should apply to them. Application of these restrictions could, for example, mean that scientific research with human embryo-like structures is prohibited in countries where research with (cloned) human embryos is either legally banned or legally restricted to the use of supernumerary human embryos (that is, human embryos that were donated for research after having been left over from medically assisted fertility treatments). In addition, it is unclear whether and how the internationally known 14-day rule (which prohibits research on human embryos from the fourteenth day of their development) can be applied sensibly to structures whose development is not synchronous to that of human embryos of the same age.

The second part of this research focused on the empirical validation and complementation of these questions and findings. How do laypeople (citizens) and 'normative professionals' (ethicists and lawyers, but also respondents reasoning from particular (non-)religious worldview perspectives) view these developments? Are there perhaps questions or concerns that we might have missed? To explore these issues, both focus groups (three with citizens and one with ethicists and lawyers) and individual interviews (with five respondents who could reflect on these developments from a Catholic, Protestant, Jewish, Islamic, and Humanist perspective) were held between August 2020 and May 2021. The analysis of the resulting data led to the identification of four overarching themes: two on (the gradations of and conditions for) confidence in scientific research with human-like embryo structures, and two on the question of how to (conceptually and morally) conceive of human-like embryo structures.

The analysis of the first two themes showed that positive, ambivalent, and negative perspectives on research with human embryonic-like structures were present in all focus groups, but that professionals (ethicists and lawyers) had greater confidence in existing regulatory mechanisms and were less concerned about the abuse of scientific freedom for societally undesirable goals than lay citizens were. Concerns about the application of human embryo-like structures for commercial purposes were also found in all groups but played a larger role in focus groups with lay citizens. Concerns about the (hypothetical) reproductive application of human embryonic-like structures played an equally large role in all groups. This is a notable finding because it was not expected that the potential reproductive use of these structures would play such a dominant role in the discussions:

apparently, the repeated emphasis on the distinctly non-reproductive character of research with human embryonic-like structures was insufficient to reassure participants about reproductive applications. Overall, these findings suggested that professional and lay participants considered three criteria to be important to have (greater) confidence in (the regulation of) research with human embryo-like structures. These criteria consisted of (1) regulating the scope of research with human embryo-like structures (particularly, restricting commercial purposes and prohibiting reproductive applications), (2) avoiding the development of morally relevant (or at least, morally sensitive) features in these structures (such as a beating heart, the potential to become persons, and the formation of a central nervous system), and (3) ensuring that research with these structures is developed for and in consultation with society. The analysis of the themes related to how human embryo-like structures are (conceptually and morally) perceived did not provide a clear-cut answer to the question of whether and how they should be distinguished from human embryos. On a conceptual level, traditional criteria such as 'fertilization' or 'developmental potential' were seen as determining whether to speak of an embryo. On a moral level, human embryo-like structures were generally considered to be of little worth if they lacked the characteristics that the participants considered morally relevant. These characteristics included a beating heart, consciousness and/or the ability to feel pain, and (as the main criterion) the potential to become persons. These results suggest that most participants, including participants reasoning from particular (non-)religious worldviews, did not immediately equate human embryo-like structures to human embryos. Furthermore, these findings suggest that laypeople are well able to consider the scientific use of human embryo-like structures from an ethical perspective, as demonstrated by the range of arguments and positions these participants put forth, which closely align with the range of arguments and positions found in the ethical literature.

The third and final part of this study focused on what emerged as a core concept in previous parts: the potential to become persons (which in ethical literature is referred to as the 'potentiality concept' or the 'Argument from Potential' (AfP)). In previous parts, this concept was found to play a role in two relevant contexts: that of definitions and that of the moral acceptability of research. Even though maintenance of this concept in embryo definitions can lead to problematic implications (including the exclusion of entities for which it cannot be determined whether they have that potential, and of entities for which it is clear that they do not have it but that may nevertheless deserve a certain degree of (symbolic) moral value), the concept of potentiality can be difficult to do without when it comes to the moral acceptability of research with such-like entities. Anyone who wants to explain why human embryos deserve protection while other human cells do not must somehow refer to the fact that a fully developed human person can only develop from an embryo—and anyone who wants to explain why that protection should also extend to (certain) human embryo-like structures will have to rely on that same reasoning. The assumption in both cases is that the potential to become a person confers (a certain

degree of) protection. The protection owed to the bearer of that potential is not owed due to the importance others attach to it (extrinsic value), but due to the inherent value that that potential itself confers (intrinsic value or "moral status"). But this reasoning remains contested in ethical literature: why would the possibility of becoming a human person have any (intrinsic) moral significance? For critics, this view would also imply having to grant moral status to isolated gametes and perhaps even any individual cell that could be genetically modified to develop into a human being (as became possible with Somatic Cell Nuclear Transfer (SCNT) in the past and now seems possible through the induction of specialized cells into a pluripotent state). According to these critics, these implications would be so absurd that it leaves us no other option but to throw the AfP in the trash.

According to defenders of the AfP, this criticism is based on a misinterpretation of the argument. If all it referred to were mere "possibility" that is entirely determined by contingent and external factors ('passive' potential), then it would indeed be unclear why that potential would have any moral significance. What advocates of the argument mean by potential is rather an 'active' orientation towards the realization of an intrinsic predisposition ('active' potential), which implies an autonomous and identity-preserving development: the developing embryo can only have active potential if it can (1) develop autonomously and (2) be identified as the same individual as the later child that will develop from it. So understood, it is less strange that this potential can be conceived to confer (intrinsic) moral value. Still, it is possible to distinguish between different versions of the argument. An important distinction can first be made between the full or limited moral status that different versions of the AfP confer. Full moral status refers to the protection afforded to human persons and which prevents us from treating them as mere means. On accounts in which the potential to become persons confers full moral status, potential persons (i.e., entities with 'active' potential) must thus be treated in the same way as actual (or paradigmatic) persons (like the reader). Let us call this the "Full Version of the AfP" (or "Full AfP"). Not all advocates of the AfP uphold this Full variant, however: for some, the potential to become persons can only grant limited moral status because that potential is per definition not actual yet. Let us call this the "Limited Version of the AfP" (or "Limited AfP"). The intuition that the potential of human embryos to develop into human beings (that is, paradigmatic persons) bears moral significance can thus apparently leave room for different moral conclusions, depending on the emphasis placed on the continuity (Full AfP) or the discontinuity (Limited AfP) between what the embryo currently is and what it has the potential to become. A second difference between versions of the AfP concerns the question of when active potential can be attributed. As mentioned earlier, active potential requires not only that an organism develops autonomously, but also that it maintains its identity throughout that process. According to some advocates of the argument, this is already the case at conception, while others argue that the fact that embryos can split or fuse until the beginning of gastrulation (which begins at around fourteen days after fertilization) must mean that development cannot be identity

preserving before gastrulation. Let us call this the individuation criterion. According to advocates of the individuation criterion, pre-gastrulation embryos (and human embryo-like structures) thus cannot (yet) have active potential.

Based on these two distinctions, it becomes possible to distinguish between four different versions of the AfP: full moral status from conception or individuation ('C-Full AfP' or 'I-Full AfP'), and limited moral status from conception or individuation ('C-Limited AfP' or 'I-Limited AfP'). Which version is adopted, is of direct relevance for the regulation of research with potential persons (whether embryos or embryo-like structures). The C-Full AfP means that there can be no room for instrumental (let alone destructive) research with potential persons, while the I-Full AfP implies that there can be no good reason to restrict research before gastrulation (at least, not based on the concept of active potential). The embryo legislation enforced in most countries, including the Dutch Embryo Act, does impose such restrictions: early human embryos can only be used for research under strict (aforementioned) conditions of proportionality and subsidiarity. In terms of the AfP, this type of legislation can thus only be justified in terms of the C-Limited AfP. The I-Limited AfP variant holds that restrictions on research with potential persons can only be imposed after fourteen days (when splitting and fusion are no longer possible). The current 14-day rule as a limit after which research with potential persons is no longer possible can only be defended based on the I-Full AfP, and not on any of the Limited AfP variants.

However, the debate about the sustainability of the AfP is still far from settled. Critics argue that research with human embryo-like structures definitively shows that embryology has the character of a mechanical box that contains all kinds of possibilities whose realization is entirely dependent on external factors, like bringing together particular types of stem cells under certain conditions. A lot hinges on whether the AfP can withstand this criticism: if all potentials are a mere possibility ('passive'), then the AfP loses its foundation in any variant. The question then arises as to what the basis can be for attributing moral status to potential persons (such as embryos and equivalent human embryo-like structures) and therefore for imposing conditions and restrictions on their use in research. Since there are no other (intrinsically) morally relevant properties available in early embryonic development, it would seem that, without the AfP, there can be no alternative grounds for such restrictions. For advocates of the AfP, however, human embryo-like structures show that autonomous and identity-preserving ('active') development is not possible in every group of human cells, and therefore that the aforementioned criticism does not have to be a final blow to the AfP or the legislations based on it. If we assume for the sake of debate that these advocates are right, then the question becomes what this should mean for the regulation of scientific research with human embryo-like structures. At what point is identity preservation possible in these structures? Which steps in their laboratory culture can and cannot be considered 'potentiality switches'? A new question in comparison to the traditional debate is, for

example, how to conceive of human embryo-like structures that contain the cells of the embryo proper but not those of the extra-embryonic tissues (from which, for example, the placenta arises), such as gastruloids. If we suppose that it might become possible to enable such 'incomplete' human embryo-like structures to develop further by using hypothetical support and cultivation techniques, should this then be seen as 'switching on active potential' or would it be more appropriate to compare it to placing an embryo in a receptive uterus? If the latter case, then the absence of the extra-embryonic tissue does not necessarily imply that such structures lack active potential.

Research into the conditions under which autonomous development occurs may provide insight into how the process of 'active potential' begins and how it can be triggered, but as long as there is insufficient knowledge about this, it is unclear at what point research is being conducted with material that may have (a certain degree of) moral status. Moreover, any talk of active potential in the context of research with human embryo-like structures remains hypothetical until we know whether (improved) structures can develop into human beings. Taken together, these considerations provide an argument for 'precaution': some commentators have argued on grounds of 'pragmatic consistency' that research with human embryo-like structures that possess all of the components of human embryos generated by fertilization (including extra-embryonic tissues) should be regulated in the same way as research with those embryos. This approach is also reflected in the recently updated guidelines of the International Society for Stem Cell Research (ISSCR), the international association of stem cell researchers, which recommend subjecting research with human embryo-like structures that attempt to model the integrated (or 'complete') development of embryos to stricter conditions (in terms of ethics review) than research with structures that do not. There is much to be said for such a precautionary approach, especially if it is explicitly justified in terms of the AfP. Nonetheless, important questions and uncertainties remain. For example, it has been suggested in the literature that using genetic modification to ensure that human embryo-like structures cannot develop beyond a predetermined stage (and therefore effectively cannot develop into persons) could function as one such precautionary measure. However, this does require that the modification be built in preventively, that is, before developmental stages at which there may already be active potential. According to the analysis outlined earlier, such a preventive modification step could be acceptable for advocates of the AfP, except for those who adhere to the C-Full AfP specifically (according to this variant, such a modification step would merely amount to creating a person with an intentionally shortened lifespan). In all other versions of the AfP, such a preventive modification step can be used to prevent the creation of an entity with active potential (and corresponding moral status), but for that, this modification step must lead to an internal (rather than external) obstruction of developmental potential. That is certainly the case if the modification intervenes in the development of the cells that will form the embryo proper. However, in light of the earlier discussion about the type of potential of human

embryo-like structures that lack extra-embryonic tissues (such as gastruloids), it may be defensible to argue that a genetic modification step that only prevents implantation would not be sufficient to prevent the emergence of active potential.

To conclude, this research study underscored that research with (different types of) human embryo-like structures (and human embryos) can be ethically justified, but that this does require adjusting contemporary policies and regulations. The extent of these adjustments and the conditions they should stipulate depend on the structures in question: human embryo-like structures are a heterogeneous group and not all research in this area is intended to replicate the integrated development of a 'complete' human embryo. Human embryo-like structures that only model part of the embryonic and/or extra-embryonic tissues do not have the developmental potential of human embryos and their use in research should therefore remain outside the scope of embryo regulations (which does not mean it should be excluded from ethics review, as these structures might still raise certain moral sensitivities). When using human embryo-like structures that come closer to modeling the integrated development of human embryos, it cannot be ruled out that they will at some point acquire the same developmental potential as those embryos, and that their use as research material will have to be subject to the same restrictions. Even though a (not ruled out) potential to develop into persons can be seen as a *prima facie* reason for precautionary measures, it should be remembered that this reasoning ultimately rests on the AfP, which is not only disputed but also open to various interpretations (especially regarding the onset of that potential and what it implies for moral status). Some of the recommendations made based on this research study, such as revising the definition of the human embryo to include a subset of human embryo-like structures under the scope of the law and lifting the ban on the special creation of human embryos for research, have already made their way into the current policy debate, including in the context of Rutte IV cabinet's proposed revision of the Dutch Embryo Act.

PUBLIEKSSAMENVATTING

Wetenschappers zijn erin geslaagd om door (verschillende soorten) pluripotente stamcellen bijeen te brengen celstructuren te kweken die op menselijke embryo's in bepaalde fases van de vroege ontwikkeling lijken. Deze zogenoemde 'humane embryoachtige structuren' zouden een (volgens sommigen, ethisch en juridisch neutraal) alternatief kunnen bieden voor het gebruik van menselijke embryo's in wetenschappelijk onderzoek. Bijvoorbeeld omdat ze op grote schaal geproduceerd kunnen worden zonder dat daarvoor invasieve procedures (eiceldonatie) nodig zijn en omdat ze niet onder de wet- en regelgeving vallen die voor onderzoek met menselijke embryo's geldt. Bovendien is het bij gebruik van deze structuren mogelijk om bepaalde elementen toe te voegen of weg te laten, waardoor de vroege menselijke ontwikkeling voor het eerst vanuit een bottom-up benadering bestudeerd kan worden. Het doel van het in dit boek beschreven onderzoek was daarom om na te gaan of en, zo ja, onder welke voorwaarden wetenschappelijk onderzoek met humane embryoachtige structuren inderdaad een ethisch aanvaardbaar alternatief kan bieden voor het wetenschappelijk gebruik van menselijke embryo's.

Het eerste deel van dit onderzoek richtte zich op het verkennen van de verschillende soorten (humane) embryoachtige structuren en de mogelijke conceptuele, ethische en juridische vraagstukken die het gebruik ervan in wetenschappelijk onderzoek zou kunnen roepen. In dit deel van het onderzoek bleek dat, hoewel de meeste embryoachtige structuren nog altijd uit dierlijke stamcellen gekweekt worden, er inmiddels ook verschillende humane varianten gemaakt zijn (zoals 'blastoïden', 'gastruloïden' en 'Post-Implantation Amniotic Sac Embryoids' (PASE)). Steeds gaat het om een geheel van cellen waarvan de organisatie en differentiatie lijkt op die van een menselijk embryo in een bepaalde fase van de vroege ontwikkeling. 'PASE' recapituleren meerdere gebeurtenissen rond de ontwikkeling van de vruchtzak. 'Gastruloïden' lijken op het 'eigenlijke embryo' in het stadium van de gastrulatie (dat na ca. twee weken begint met de vorming van de primitiefstreep) en missen de aanleg van de extra-embryonale weefsels (zoals de placenta) die nodig zijn voor de innesteling en de verdere ontwikkeling in de baarmoeder. 'Blastoïden' lijken op embryo's in het blastocyste stadium (ca. 5 dagen in ontwikkeling) en bestaan uit alle celtypen voor verdere ontwikkeling: zowel die van het 'eigenlijke embryo', als die van de extra-embryonale weefsels. Voor de nog vroegere embryonale ontwikkeling bestaan (nog) geen overeenkomstige embryoachtige structuur, hoewel onderzoek met recent ontdekte 'Extended Pluripotent' stamcellen daar misschien verandering in kan brengen. De zogeheten 'ETS/X-embryo's', welke eveneens uit embryonale en extra-embryonale weefsels bestaan, zijn (nog) niet uit menselijke stamcellen gekweekt maar lijken in muizen de ontwikkeling te kunnen modelleren vanaf het blastocyste stadium tot aan de (vroege) organogenese (tussen ca. dag 5.5 en 8.5). Op dit moment zijn alle structuren gebrekkig en hebben ze een beperkt ontwikkelingspotentieel, maar er wordt

wereldwijd gewerkt aan verbetering. Die verdere verbetering maakt het denkbaar dat met wat bijvoorbeeld begint als humane blastoïden ook de ontwikkeling van menselijke embryo's in latere stadia kan worden gerepliceerd en bestudeerd. Hoewel het niet voor alle onderzoeksvragen nodig is om de volledige embryonale ontwikkeling te repliceren, lijkt het dus te verwachten dat dit in de toekomst steeds beter mogelijk zal worden. Dat leidt onvermijdelijk tot de spannende vraag hoe dan nog kan worden onderscheiden tussen enerzijds structuren die niet meer dan modellen zijn en anderzijds dermate perfecte replica's dat eigenlijk sprake is van uit stamcellen gegenereerde menselijke embryo's. De paradox die dit oplevert is dat hoe beter humane embryoachtige structuren worden, des te lastiger het wordt om ze als een ethisch en juridisch neutraal alternatief voor het gebruik van menselijke embryo's te beschouwen. Waar die overgang precies ligt is niet makkelijk te beantwoorden: terwijl in dieronderzoek de geboorte van gezond (en vruchtbaar) nageslacht als proef op de som kan worden beschouwd, is dat bij onderzoek met uit humane cellen tot stand gebrachte embryoachtige structuren om ethische redenen niet mogelijk.

Deze verkenning mondde uit in het agenderen van vragen voor verder onderzoek op conceptueel-, moreel- en beleidsniveau. Aangezien er geen universeel aanvaarde definitie van menselijke embryo's bestaat, zijn verschillende antwoorden mogelijk op de conceptuele vraag of (bepaalde) humane embryoachtige structuren wel of niet als embryo's te beschouwen zijn. Aangezien geen van deze structuren voortkomt uit de fusie van gameten, is het waarschijnlijk dat ze bij voorbaat niet als embryo's kunnen worden beschouwd in landen waar de bevruchting als noodzakelijke voorwaarde van embryodefinities beschouwd wordt (zoals dat bijvoorbeeld het geval is in Spanje). Of ze als embryo's te beschouwen zijn in landen waar de nadruk van embryodefinities op ontwikkelingspotentieel (oftewel: het vermogen om de ontwikkeling voort te zetten) ligt is minder duidelijk. Als de nadruk op het initiëren van menselijke ontwikkeling ligt (zoals dat het geval is in Australië) is het waarschijnlijk dat slechts een subset van humane embryoachtige structuren als embryo's moeten worden beschouwd. Welke subset dat is, hangt af van de stand van de techniek. Maar als die nadruk op het vermogen uit te groeien tot mens ligt (zoals dat het geval is in België en Nederland en waarbij het zou moeten gaan om organismen die hun ontwikkeling naar verwachting zullen voortzetten tot aan minstens de geboorte) wordt het nog uitdagender om aan te wijzen welke structuren wel of niet als zodanig beschouwd kunnen worden: zoals eerdergenoemd, is het simpelweg niet mogelijk om op ethisch aanvaardbare wijze te experimenteren of humane embryoachtige structuren wel of niet dat vermogen hebben. Dat leidt tot een epistemologische uitdaging. De conceptuele vraag of humane embryoachtige structuren wel of niet als embryo's te beschouwen zijn moet echter wel onderscheiden worden van de morele vraag of en in hoeverre ze bescherming verdienen. Als (bepaalde) humane embryoachtige structuren eigenschappen bezitten die als moreel relevant beschouwd kunnen worden (zoals bijvoorbeeld het vermogen om pijn te lijden, het vermogen

om mens te worden, of beginnende hersenactiviteit) dan kan een zekere mate van beschermwaardigheid gerechtvaardigd zijn ongeacht of ze embryo's zijn. Als humane embryoachtige structuren bijvoorbeeld hetzelfde vermogen om mens te worden hebben als menselijke embryo's, dan moet dat betekenen dat ze dezelfde morele status (en dus dezelfde beschermwaardigheid) moeten hebben als menselijke embryo's die vanwege dat vermogen beschermd worden. De vraag blijft echter op grond waarvan dat vermogen morele betekenis kan verlenen; die vraag wordt verderop in het onderzoek nader uitgediept. Beleidsmatig roepen deze bevindingen enkele specifieke uitdagingen op. Aan de ene kant, als aangenomen wordt dat (bepaalde) humane embryoachtige structuren niet als embryo's te beschouwen zijn (bijvoorbeeld omdat ze niet het vermogen hebben om mens te worden), zal het wetenschappelijk gebruik ervan alleen onderworpen hoeven worden aan de (minder strikte) regels die voor onderzoek met menselijke cellen en weefsels in het algemeen gelden. Vanuit een subsidiariteitsperspectief zou dit tevens kunnen betekenen dat het gebruik van deze humane embryoachtige structuren voorrang zou moeten krijgen boven het gebruik van dieren en menselijke embryo's in wetenschappelijk onderzoek. Aangezien het echter denkbaar is dat humane embryoachtige structuren morele gevoeligheden kunnen oproepen ongeacht of ze wel of geen embryo's zijn, is hier mogelijk sprake van een beschermingslacune. Aan de andere kant, als (verbeterde) humane embryoachtige structuren wél als embryo's kunnen worden beschouwd, wordt de vraag of en hoe de beperkingen die gelden voor menselijk embryo-onderzoek ook op het wetenschappelijk gebruik van dergelijke embryoachtige structuren moeten worden toegepast. Toepassing van deze beperkingen zou bijvoorbeeld kunnen betekenen dat wetenschappelijk onderzoek met humane embryoachtige structuren verboden wordt in landen waar onderzoek met (gekloneerde) embryo's ofwel wettelijk verboden is, ofwel wettelijk beperkt wordt tot het gebruik van restembryo's (dat wil zeggen embryo's die overgebleven zijn na fertiliteitsbehandelingen en die door de donoren voor wetenschappelijk onderzoek beschikbaar zijn gesteld). Bovendien is het onduidelijk of en hoe de internationaal bekende 14-dagenregel (die onderzoek op menselijke embryo's verbiedt vanaf de veertiende dag van hun ontwikkeling) zinvol toegepast kan worden op structuren waarvan de ontwikkeling niet synchroon loopt met die van menselijke embryo's van dezelfde leeftijd.

Het tweede deel van dit onderzoek richtte zich op het empirisch toetsen en aanvullen van deze vragen en bevindingen. Hoe kijken leken (burgers) en 'normatieve professionals' (ethici en juristen maar ook respondenten vanuit levensbeschouwelijk perspectief) naar deze ontwikkelingen? Zijn er misschien vragen of zorgen die we gemist hebben? Om deze zaken te verkennen zijn zowel focusgroepen (waarvan drie met burgers en een met ethici en juristen) alsook individuele interviews (met vijf respondenten die vanuit Rooms-Katholiek, Protestant, Joods, Islamitisch en Humanistisch perspectief konden reflecteren op deze ontwikkelingen) gehouden tussen augustus 2020 en mei 2021. De analyse van de hieruit voortvloeiende uitkomsten resulteerde in de identificatie van vier

overkoepelende thema's: twee over (gradaties van en voorwaarden voor) vertrouwen in wetenschappelijk onderzoek met humane embryoachtige structuren en twee over de vraag hoe (conceptueel en moreel) wordt aangekeken tegen humane embryoachtige structuren.

Uit de analyse van de eerste twee thema's bleek dat positieve, ambivalente en negatieve opvattingen over wetenschappelijk onderzoek met humane embryoachtige structuren in alle focusgroepen aanwezig waren, maar dat professionals (ethici en juristen) een groter vertrouwen hadden in bestaande regelgevende mechanismen en minder bezorgd waren over het misbruik van wetenschappelijke vrijheid voor maatschappelijk ongewenste doelen dan burgers. Bezorgdheid over toepassingen voor commerciële doelen werden bij alle focusgroepen gevonden maar speelde vooral een grote rol in de focusgroepen met burgers. Zorgen om het (vooralsnog, hypothetisch) reproductief gebruik van humane embryoachtige structuren speelde bij alle groepen een even grote rol. Dit is een opmerkelijke bevinding omdat er niet van tevoren verwacht werd dat het mogelijk reproductief gebruik van deze structuren zo'n dominante rol in de discussies zou hebben: het meermaals benadrukken dat onderzoek met humane embryoachtige structuren een nadrukkelijk niet-reproductief karakter heeft, was kennelijk onvoldoende geruststellend om zorgen over reproductieve toepassingen bij deelnemers weg te nemen. Alles overziend, wezen deze bevindingen erop dat de professionals en lekendeelnemers drie criteria belangrijk vonden om (meer) vertrouwen te hebben in (de beleidsvorming voor) wetenschappelijk onderzoek met humane embryoachtige structuren. Deze criteria waren (1) het reguleren van het toepassingsgebied van wetenschappelijk onderzoek met humane embryoachtige structuren (in het bijzonder het inperken van commerciële doelen en het verbieden van reproductieve toepassingen), (2) het vermijden van het doen ontstaan van moreel relevante (of in ieder geval, moreel gevoelige) eigenschappen in deze structuren (waarbij onder meer gedacht werd aan een kloppend hart, het vermogen mens te worden en de vorming van een centraal zenuwstelsel), en (3) het waarborgen dat wetenschappelijk onderzoek met deze structuren in samenspraak met en voor de maatschappij ontwikkeld wordt. De analyse van de thema's die betrekking hadden op de vraag hoe humane embryoachtige structuren in conceptueel en moreel opzicht moeten worden gekwalificeerd gaf geen eenduidig antwoord op de vraag of en hoe ze wel of niet onderscheden moeten worden van menselijke embryo's. Op conceptueel niveau werden traditionele criteria als 'bevruchting' of 'ontwikkelingspotentieel' als bepalend gezien om wel of niet van een embryo te spreken. Op moreel niveau werden humane embryoachtige structuren over het algemeen als weinig beschermwaardig geacht indien de volgens de deelnemers moreel relevante eigenschappen ontbraken. Eigenschappen die als moreel relevant geacht werden waren onder andere een kloppend hart, bewustzijn en/of het vermogen om pijn te lijden, en (als belangrijkste criterium) het vermogen mens te worden. Uit het geheel van deze resultaten is op te maken dat de meeste deelnemers, waaronder deelnemers die vanuit specifieke levensbeschouwelijke perspectieven redeneerden, de

neiging hadden om embryo's en embryoachtige structuren niet zonder meer aan elkaar gelijk te stellen. Bovendien wijzen deze bevindingen erop dat leken goed in staat zijn om het wetenschappelijk gebruik van humane embryoachtige structuren vanuit ethisch perspectief te beschouwen. Dat blijkt uit het spectrum aan argumenten en standpunten die deze deelnemers naar voren brachten en de mate van overeenstemming met het spectrum aan argumenten en standpunten die in de ethische literatuur gevonden worden.

Het derde en laatste deel van dit onderzoek besteedde aandacht aan wat in de eerdere delen als kernbegrip naar voren is gekomen: het vermogen om mens te worden (in de ethische literatuur aangeduid als het 'potentialiteitsbegrip' of 'potentialiteitsargument'). In die eerdere delen bleek dit begrip in twee relevante contexten een rol te spelen: die van definities en die van de morele aanvaardbaarheid van wetenschappelijk onderzoek. Hoewel het hanteren van het potentialiteitsbegrip in embryodefinities tot problematische implicaties kan leiden (waaronder het uitsluiten van entiteiten waarbij niet vastgesteld kan worden of ze dat vermogen hebben alsook van entiteiten waarbij het wel duidelijk is dat ze dat vermogen ontbreken maar die mogelijk desalniettemin een zekere mate van (desnoods 'symbolische') waarde verdienen), kan het potentialiteitsbegrip waar het gaat om de morele aanvaardbaarheid van onderzoek met dergelijke entiteiten moeilijk worden gemist. Wie wil kunnen uitleggen waarom menselijke embryo's wél en andere menselijke cellen géén bescherming toekomt, ontkomt er namelijk niet aan op de een of andere manier te verwijzen naar het feit dat uit zo'n embryo een volledig ontwikkeld mens kan groeien—en wie wil kunnen uitleggen waarom die bescherming zich tevens zou moeten uitstrekken tot (bepaalde) humane embryoachtige structuren zal zich van diezelfde redenering moeten bedienen. De veronderstelling is in beide gevallen dat het vermogen mens te worden (een zekere mate van) beschermwaardigheid verleent. De beschermwaardigheid die aan de bezitter van dat vermogen verschuldigd is, is niet verschuldigd vanwege het belang dat anderen aan dat vermogen hechten (extrinsieke waarde), maar vanwege de inherente waarde dat dat vermogen op zichzelf heeft (intrinsieke waarde of 'morele status'). Toch wordt deze redenering in de ethische literatuur betwist: waarom zou de mogelijkheid om mens te worden (intrinsieke) morele betekenis moeten hebben? Wie dat serieus meent, aldus critici, zou immers ook morele status moeten toekennen aan afzonderlijke geslachtscellen en misschien zelfs aan individuele lichaamscellen die er via genetische modificatie toe zouden kunnen worden gebracht om uit te groeien tot een mens (zoals dat vroeger al mogelijk was met celkerntransplantatie en nu ook mogelijk lijkt te zijn door somatische cellen te induceren in een pluripotent stadium). Volgens critici zouden deze implicaties dusdanig absurd zijn dat haast niets anders mogelijk is dan het potentialiteitsargument in de prullenbak te gooien.

Volgens verdedigers van het potentialiteitsargument berust deze kritiek op een onjuiste interpretatie van potentialiteit. Als daarmee een loutere 'mogelijkheid' bedoeld zou worden waarvan de realisering geheel en al wordt bepaald door contingente en

externe factoren ('passieve potentialiteit'), dan zou het inderdaad onduidelijk zijn waarom die potentialiteit morele betekenis zou moeten hebben. Met potentialiteit bedoelen verdedigers van het argument echter een 'actieve' gerichtheid op de realisering van een intrinsieke bestemming ('actieve potentialiteit') en dat veronderstelt een autonome en identiteitsbehoudende ontwikkeling: het zich ontwikkelende embryo kan alleen een actief vermogen hebben om mens te worden als (1) het zich autonoom kan ontwikkelen en (2) het geïdentificeerd kan worden als hetzelfde individu als het latere kind dat eruit ontstaat. Zo opgevat is het minder vreemd dat aan dit vermogen (intrinsieke) morele betekenis kan worden gehecht. Toch is het mogelijk om tussen verschillende versies van deze redenering te onderscheiden. Een belangrijk onderscheid valt om te beginnen te maken tussen versies van het potentialiteitsargument die uitkomen bij een volledige dan wel een beperkte morele status. Onder volledige morele status wordt verstaan de status die ieder menselijke persoon toekomt en die ons weerhoudt om personen louter als middel te behandelen. Als volledige morele status verbonden wordt aan het vermogen mens te worden moeten potentiële personen (oftewel, entiteiten met het vermogen mens te worden) dus op dezelfde manier behandeld worden als actuele (of paradigmatische) personen (zoals de lezer). Laten we dit het 'Volledige Potentialiteitsargument' (of 'VP') noemen. Toch het VP niet door alle verdedigers van het potentialiteitsargument aanvaard: voor sommigen kan aan het vermogen mens te worden hoogstens beperkte morele status verlenen omdat dat vermogen per definitie nog niet gerealiseerd is. Laten we dit het 'Beperkte Potentialiteitsargument' (of 'BP') noemen. De morele intuïtie dat aan het vermogen van menselijke embryo's om uit te groeien tot een mens (paradigmatische persoon) morele betekenis moet worden toegekend laat dus kennelijk ruimte voor verschillende morele conclusies, afhankelijk van de nadruk die men wil leggen op de continuïteit ('VP') dan wel de discontinuïteit ('BP') tussen wat het embryo nu is en de persoon die daar later uit kan ontstaan. Een tweede verschil tussen versies van het potentialiteitsargument betreft de vraag vanaf welk moment actieve potentialiteit kan worden toegeschreven. Zoals eerder gezegd, moet hiervoor niet alleen sprake zijn van een organisme dat een autonome ontwikkeling doormaakt, maar ook van een organisme die zijn identiteit behoudt in dat proces. Volgens sommige verdedigers van het potentialiteitsargument is dat al vanaf de conceptie het geval, volgens andere betekent het feit dat embryo's zich nog tot aan het begin van de gastrulatie (ongeveer veertien dagen vanaf de bevruchting) kunnen splitsen of fuseren dat aan die eis van identiteitsbehoud voorafgaand aan gastrulatie niet kan worden voldaan. Laten we dit het individuatiecriterium noemen. Volgens verdedigers van het individuatiecriterium kunnen pre-gastrulatie embryo's (en humane embryoachtige structuren) dus (nog) géén actieve potentialiteit hebben.

Op basis van deze twee onderscheidingen wordt het mogelijk om tussen vier verschillende versies van het potentialiteitsargument te onderscheiden: volledige morele status vanaf de conceptie dan wel vanaf de individuatie (C-VP of I-VP), en beperkte

morele status vanaf de conceptie dan wel vanaf de individuatie (C-BP of I-BP). Welke versie aangehouden wordt is van direct belang voor de regulering van wetenschappelijk onderzoek met potentiële personen (zij het embryo's of embryoachtige structuren). C-VP betekent dat er voor verbruikend onderzoek met potentiële personen geen enkele ruimte kan zijn, terwijl I-VP impliceert dat er tot aan de gastrulatie geen goede redenen kunnen zijn om aan dat onderzoek beperkingen op te leggen (althans niet op basis van het potentialiteitsargument). De in de meeste landen geldende embryowetgeving, waaronder de Nederlandse Embryowet, doet dat wel: menselijke embryo's mogen uitsluitend onder strikte (eerdergenoemde) voorwaarden van proportionaliteit en subsidiariteit voor wetenschappelijk onderzoek worden gebruikt. In termen van het potentialiteitsargument valt dergelijke wetgeving alleen te verantwoorden in termen van C-BP. De variant I-BP houdt in dat er pas na ongeveer veertien dagen (als splitsing en fusie niet langer mogelijk zijn) voorwaarden aan onderzoek met potentiële personen te stellen zijn. De huidige 14-dagen grens als limiet waarna er in het geheel geen onderzoek met potentiële personen meer mogelijk is, valt alleen op grond van I-VP te verdedigen en dus niet op grond van een van de BP varianten.

Toch is het debat over de houdbaarheid van het potentialiteitsargument nog zeker niet beslecht. Volgens critici maakt juist onderzoek met humane embryoachtige structuren eens en voorgoed duidelijk dat de embryologie het karakter van een meccanodoos heeft die allerlei mogelijkheden in zich bergt waarvan de realisering geheel afhankelijk is van externe factoren, zoals het onder bepaalde omstandigheden bij elkaar brengen van bepaalde stamcellen. Van de vraag of het potentialiteitsargument tegen deze kritiek te verdedigen valt hangt veel af: als alle potentialiteit louter mogelijkheid ('passief') is, dan verliest het potentialiteitsargument zijn onderbouwing in welke variant dan ook. De vraag rijst dan wat dan eigenlijk de grondslag kan zijn van het toeschrijven van morele status aan potentiële personen (zoals embryo's en equivalente humane embryoachtige structuren) en dus van het opleggen van voorwaarden en beperkingen aan hun wetenschappelijk gebruik in verbruikend onderzoek. Aangezien er geen andere (intrinsiek) moreel relevante eigenschappen mogelijk lijken de zijn in de vroege embryonale ontwikkeling lijkt er, anders dan het potentialiteitsargument, geen alternatieve onderbouwing mogelijk te zijn voor dergelijke beperkingen. Volgens verdedigers van het potentialiteitsargument wijzen humane embryoachtige structuren er echter juist op dat autonome en identiteitsbehoudende ('actieve') ontwikkeling niet in alle groepen menselijke cellen mogelijk is en dus dat de eerdergenoemde kritiek helemaal geen genadeklap voor het potentialiteitsargument en daarop gebaseerde wetgeving hoeft te zijn. Als omwille van debat gesteld wordt dat deze verdedigers gelijk hebben, wordt de vraag wat dat dan moet betekenen voor de regulering van wetenschappelijk onderzoek met humane embryoachtige structuren. Vanaf welk moment is identiteitsbehoud in deze structuren mogelijk? Welke kweekstappen kunnen wel en niet beschouwd worden als 'aanzetknoppen' voor actieve potentialiteit? Een ten opzichte van het klassieke debat

nieuwe vraag is bijvoorbeeld hoe aangekeken moet worden tegen humane embryoachtige structuren die wel de cellen bevatten van het eigenlijke embryo maar niet die van de extra-embryonale weefsels (waaruit onder meer de placenta ontstaat), zoals gastruloïden. Stel dat het mogelijk wordt om dergelijke ('incomplete') humane embryoachtige structuren met behulp van hypothetische steun- en kweektechnieken alsnog tot verdere ontwikkeling in staat te stellen, moet dat dan worden gezien als 'het aanzetten van actieve potentialiteit' of kan het beter worden vergeleken met het plaatsen van een embryo in een ontvankelijke baarmoeder? In dat laatste zou het ontbreken van de aanleg van de extra-embryonale weefsels immers niet hoeven betekenen dat dergelijke structuren geen actieve potentialiteit kan worden toegeschreven.

Onderzoek naar de voorwaarden waaronder in een groep stamcellen een autonoom ontwikkelingsproces ontstaat kan wellicht inzicht geven in hoe het proces van 'actieve potentialiteit' begint en hoe het kan worden uitgelokt, maar zolang daar onvoldoende kennis over is, is het onduidelijk wanneer onderzoek plaats vindt met materiaal dat mogelijk (een zekere) morele status toekomt. Bovendien is ieder spreken van actieve potentialiteit in de context van wetenschappelijk onderzoek met humane embryoachtige structuren hypothetisch zolang we niet weten of (verbeterde) structuren daadwerkelijk het vermogen hebben om uit te groeien tot een mens. Dat alles levert een argument op voor 'voorzorg': door sommige commentatoren is op grond van overwegingen van 'pragmatische consistentie' bepleit om onderzoek met humane embryoachtige structuren die alle componenten van door bevruchting ontstane menselijke embryo's bezitten (inclusief extra-embryonale weefsels) net zo te reguleren als die embryo's. Die benadering spreekt ook uit de recentelijk geüpdatet richtlijnen van de International Society for Stem Cell Research (ISSCR), de internationale beroepsvereniging van stamcelonderzoekers, waarin wordt bepleit om onderzoek met humane embryoachtige structuren waarin wordt getracht de geïntegreerde ontwikkeling van de volledige conceptus te modelleren aan strikere voorwaarden (in termen van medisch-ethische toetsing) te onderwerpen dan onderzoek met structuren waarbij dat niet het geval is. Voor zo'n voorzorgsbenadering valt veel te zeggen, zeker als die benadering expliciet verantwoord wordt in termen van het potentialiteitsargument. Toch zijn ook hier nog belangrijke vragen en onzekerheden. Zo wordt het bijvoorbeeld in de literatuur gesuggereerd dat gebruik maken van genetische modificatie om te bewerkstelligen dat humane embryoachtige structuren zich niet verder kunnen ontwikkelen dan een vooraf bepaald stadium (en dus niet kunnen uitgroeien tot mens) mogelijk als een dergelijk voorzorgsmaatregel kan fungeren. Dat vereist echter wel dat die modificatie preventief wordt ingebouwd, dat wil zeggen voorafgaand aan ontwikkelingsstadia waarin mogelijk al sprake is van actieve potentialiteit. Volgens de eerder uiteengezette analyse kan zo'n preventieve modificatiestap voor verdedigers van het potentialiteitsargument aanvaardbaar zijn, behalve voor verdedigers die de specifieke variant C-VP aanhouden (volgens die variant komt zo'n modificatiestap neer op het doen ontstaan van een persoon met een opzettelijk verkorte levensduur). In alle andere

versies van het potentialiteitsargument kan met zo'n preventieve modificatiestap worden bereikt dat er geen entiteit met actieve potentialiteit (en daarop gebaseerde morele status) ontstaat, maar het is dan wel zaak dat die modificatiestap leidt tot een interne (en niet tot een externe) obstructie van het verdere ontwikkelingspotentieel. Van het eerste is zeker sprake als die modificatie ingrijpt op de ontwikkeling van de cellen die het eigenlijke embryo zullen gaan vormen. Maar in het licht van de eerdere discussie over de morele betekenis van het mogelijke ontwikkelingspotentieel van humane embryoachtige structuren waarin de extra-embryonale weefsels ontbreken (zoals gastruloïden), valt wellicht te verdedigen dat een genetische modificatiestap die uitsluitend de implantatie zou verhinderen niet voldoende zal zijn om het ontstaan van actieve potentialiteit te voorkomen.

Tot slot, dit onderzoek onderstreepte dat ethisch verantwoord wetenschappelijk onderzoek met (verschillende soorten) humane embryoachtige structuren (en menselijke embryo's) mogelijk is maar dat dit wel vraagt om aanpassing van beleid en regelgeving. In welke mate en welke voorwaarden daarbij gesteld moeten worden, hangt af van de structuren in kwestie: humane embryoachtige structuren zijn heterogeen van aard en lang niet alle onderzoek op dit gebied is erop gericht de geïntegreerde ontwikkeling van een 'compleet' menselijk embryo na te bootsen. Humane embryoachtige structuren die slechts een deel van de embryonale en/of extra-embryonale weefsels modelleren, hebben niet het ontwikkelingspotentieel van menselijke embryo's en hun gebruik in wetenschappelijk onderzoek hoort daarom buiten de reikwijdte van de regelgeving voor embryo-onderzoek te blijven (wat overigens nog niet wil zeggen dat iedere vorm van medisch-ethische toetsing overbodig is want ook deze structuren kunnen bepaalde morele gevoeligheden oproepen). Bij gebruik van humane embryoachtige structuren die dichter in de buurt komen van de geïntegreerde ontwikkeling van menselijke embryo's valt niet uit te sluiten dat ze op enig moment eenzelfde ontwikkelingspotentieel verkrijgen als dergelijke embryo's en dat hun gebruik als wetenschappelijk onderzoeksmateriaal aan dezelfde beperkingen gebonden zal moeten worden. Hoewel een (niet uit te sluiten) vermogen uit te groeien tot mens kan worden beschouwd als een *prima facie* reden voor een voorzorgsbeleid, moet worden bedacht dat die redenering uiteindelijk berust op het potentialiteitsargument, dat niet alleen betwist wordt maar ook op verschillende manieren te interpreteren valt (met name waar het gaat om de vraag waar dat vermogen begint en wat eruit volgt voor morele status). Van de op dit onderzoek gebaseerde aanbevelingen voor verantwoord wetenschappelijk onderzoek met humane embryoachtige structuren, waaronder het aanpassen van de embryodefinitie om een subset van humane embryoachtige structuren onder de wet te brengen en het opheffen van het moratorium op het speciaal kweken van embryo's voor onderzoek, heeft een gedeelte al zijn weg gevonden in het huidige beleidsdebat, onder meer in de context van de door het kabinet Rutte IV beoogde aanpassing van de Nederlandse Embryowet.

VALORIZATION

Maastricht University encourages reflection upon the scientific and societal impact of a doctoral thesis in a separate paragraph. In the following section, I will therefore consider how my research has and can continue to contribute to the scientific community and to society in general. I reflect in particular upon how the results of this thesis are useful to the different stakeholders, and I describe how they have been and will continue be disseminated among professionals and laypeople.

Advancing societal and scientific debate

Research with human embryo-like structures is a rapidly emerging field of research for which there are high-stakes and very few leading guidelines. On the one hand, there is anticipation that these models can provide valuable tools to complement and perhaps even replace the use of human embryos in fundamental and clinical research. The unprecedented scalability and adaptability these models provide makes them especially useful, as it enables scientists to create and modify them as necessary for specific research aims, while simultaneously bypassing many of the practical and legal limitations associated with the use of human embryos for equivalent purposes. On the other, there is uncertainty about how these developments will be received by society in general, and about whether human embryo models indeed provide a morally unproblematic way of conducting research into early human development in particular. These uncertainties are further invigorated by the fact that traditional approaches to policymaking in associated fields of research (e.g., time-based constraints) cannot address the particular challenges human embryo models raise, and thus that blinded extension of embryo protective regulations may risk damping important scientific avenues of research for little more than a false sense of political security.

These issues are understandably prompting a great deal of societal and scientific debate. Considering that this thesis inquired whether and under what conditions research with human embryo-like strictures can be ethically acceptable, the findings it produced can thus be useful to a series of stakeholders. First, to biomedical researchers. From my talks with scientists directly involved in the development of human embryo models, I learned that most are genuinely concerned about the ethical justification of their work and especially welcoming of further normative clarity. The insights of this thesis with regard to the moral issues that particular human embryo models may raise provides first aid to researchers struggling with the ethics of their work until concrete regulations can be established. They also provide a roadmap for ethicists and policymakers involved in the development of sustainable normative frameworks for research with human embryo models by highlighting certain key issues in need of further ethical scrutiny. In fact, the issues flagged as requiring further ethical inquiry in the first article I co-authored have already been picked up at a national and international level by the experts involved

in the **Third Evaluation of the Dutch Embryo Act** (2021), and those involved in the **Updated Guidelines of the ISSCR** (2021). More recently, they also came to the attention of the institutional bodies responsible for human embryo research regulation in the Netherlands and the United Kingdom. Both of these jurisdictions are now considering if and how legislation for research with human embryos should be adapted to account for human embryo models that might become indistinguishable from them, and the work laid out in this thesis can help inform these discussions. I was therefore invited to join an expert consultation on the topic by the Dutch **Ministry of Public Health, Wellbeing and Sport** (VWS), and the British **Human Fertilisation and Embryology Authority** (HFEA). Follow-up meetings will take place soon. In the Netherlands, the results of this thesis will also be of direct relevance to the researchers involved in the **Pluripotent Stem cell for Inherited Diseases and Embryo Research** (PSIDER), a multidisciplinary research program with several consortia on research with embryo models created from induced pluripotent stem cells. Insights pertaining to the Argument from Potential, for instance, will be of direct relevance to the future post-doc research I look forward to undertake within the HipGametes consortium of PSIDER. Lastly, even though human embryo-like models are still far from clinical practice, and even further from quotidian life, the findings described in this thesis are also relevant to laypeople. The fact that these models exist challenges previous conceptions of human life, and prompts fundamental questions about moral values and moral meaning. These questions become especially pressing when these models are conceived to be used for certain purposes, like commercial and reproductive ones, or in combination with other (traditionally controversial) biotechnologies, such as gene-editing and artificial womb technologies.

Research outreach activities

The results of this thesis were disseminated through academic and non-academic channels in an effort to get the word out to as many different stakeholders as possible. Dissemination through academic channels consisted of publications in international peer-reviewed journals and presentations at international scientific conferences. At the time of writing, three of the articles I co-authored have been published (in **Human Reproduction Update** (2020), **RBMO** (2021), **Humanities and Social Sciences Communications** (2022)), one has been resubmitted to the **Journal of Bioethical Inquiry** (2022), and one has been submitted to **Medicine, Health Care and Philosophy** (2022). Aside from the regular yet informal presentations I held at MERLN throughout the years, I also presented the findings leading up to the aforementioned articles at formal scientific events, such as the virtual **TERMIS** and **Reproductive Ethics** conferences, as well as the virtual **ISSCR** and **ESHRE** annual meetings. I was invited to join as a speaker at the virtual annual conference of the **Progress Educational Trust** (2021), the online **RBMO** live webinar of the same journal and of the International IVF Initiative (2021), the first live **PSIDER** event (2022), and the **ESHRE Campus** symposium entitled "New Approaches for Understanding Early

Human Development" held in October 2022.

Considering that research with human embryo-like structures might be societally sensitive, I also undertook outreach activities to raise awareness about the topic outside the scientific community. The most significant ones to mention are of course the focus group and individual interviews I held in the context of my PhD research. To the best of my knowledge, these interviews were the first to probe the topic empirically and to provide experimental data on public perspectives toward research with human embryo-like structures, which we have made accessible to fellow researchers in an effort to promote further analysis. Other activities included giving an invited talk at the 24th edition of the Dutch philosophical **Talkshow De Idee** (2019), which is also available as a podcast, writing columns about my research findings for **RegMed XB** (2020), and contributing to a piece on the topic by the Dutch **New Scientist** (2022). In my most recent attempt to further increase public outreach, I also inquired at **NEMOKennislink** whether it would be of interest to write on the topic, which was well received and is ongoing.

ACKNOWLEDGMENTS

Getting to write this section as a first-generation immigrant is a cherry on top of a longer and unlikely journey whose significance I feel compelled to acknowledge here. That journey began roughly half a life ago, on June 23, 2008, as my fourteen-year-old self cautiously stepped off a bus and into the unknown to reunite with her family. It took many more steps to get from that place to where I stand today: very few were as cautious and even fewer were as pivotal, yet all had tremendous meaning to me because of how fortunate I was to get to set them in the company of people I admire. It is thanks to these people that this journey has been possible and, more importantly, worth the while. In this section, my aim is therefore to put them in at least an inch of the limelight they truly deserve.

I feel incredibly privileged to have had so many committed and insightful teachers throughout my student years. Teachers I look up to because they believed, and through it, taught me to believe that I could take any path to Rome if I just continued walking. On that score, I am grateful to **António Cide, Marieke Bouwmeester, Jolanda Boeije, dr. ir. Geert Munnichs** and **prof. dr. Frans Brom,** for the enduring (self-)confidence they instilled in me and for the many other, more particular ways in which each of them contributed to my personal and professional development at different stages of my journey. Whenever it became difficult for me to believe I could (still) get on a path to Rome, what kept me going was to know that you did.

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hours over the phone: you are always there for me with a surprisingly new perspective or a sincerely kind word. It was already an honor to be your paranymph, but it is even more so to have you be mine. **Alena,** thank you for introducing me to a completely new and healthier version of myself (I am—quite visibly—a different person, and that is in important ways thanks to you), for constantly reminding me to "just keep taking one step at a time", for always delivering flagrant honesty in a considerate fashion, and for the many other, less visible but just as significant ways in which you have contributed to my well-being and way of life. You are truly a great friend.

Whenever my feet blistered and my legs threatened to give in, I was fortunate to have a handful of people care for me and drag me through my journey when necessary. It is a very rare thing, to be able to lean on people so confidently, and my greatest wealth is to have them walk by my side from the early beginnings. On that note, I am particularly grateful to **Roxanne van der Puil, Jonas Franzen, Careth Looijen, Vasco Graça,** and **Hakan Gargili,** and to my lifelong friends, **Mariana Pêta, Sarah al Dhahir, Joaquin Hernandez Lopez, Veselin Milev** (and **Ellen Gale**)**, Zette Zho,** and **Igor Pavlovic,** for being so unapologetically themselves, so unconditionally supportive, and so remarkably patient with me. I was not always able to be the friend you deserve over these past few years, but you have always made me feel as if I were.

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When I think of my family now, I cannot help but jokingly say that I was predestined to become an immigrant. My father, **Mikhael Hanna Daoud,** my mother, **Maria Rocha Pereira,** and their fathers and mothers, all had to flee their countries at some point in their lives in search of a chance at better ones. Yet when the time came to immigrate myself, I was too young to see the joke and much too childish to be able to appreciate it. My mom and (other) dad, **Eduardo Récio Lança,** had left for The Hague for arbitrary reasons (a job that turned out to be inexistent), and their decision to stay and make it

work nonetheless seemed completely reckless to me then. Looking back, I only wish I had been quicker to learn that the ability to *"... make one heap of all our winnings and risk it on one turn of pitch-and-toss, and lose, and start again at your beginnings and never breathe a word about your loss ..."* (as put in 'If' by Rudyard Kipling) is, in fact, an intrinsic part of being a Man—and that my parents' seemingly reckless decision was, in fact, an act of admirable courage. **Mom, dad(s),** and **little sister, Filipa Pereira Lança:** I could never have had the opportunities that made my way to writing this book if it were not for you and the sacrifices you made. I hope you know how endlessly grateful I am for your bravery, your selflessness, and your everyday example, despite however long it took me to realize it.

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Growing up, I used to be told *"diz-me com quem andas e dir-te-ei quem és"* ("tell me with whom you walk, and I shall tell you who you are"). I always found the proverb to be too presumptuous, but I think I might be starting to agree with the essence of it. Whatever the length of our walk, it has forever enriched my journey and character and, for that, I am truly and indescribably grateful.

Thank you for walking with me.

ABOUT THE AUTHOR

Ana Pereira Daoud was born in Lisbon, on April 22, 1994. As the first-born of a former fertility patient and by then single parent, she had a sheltered childhood and used to spend most of her time either drawing or reading the works of feminist poet Florbela Espanca. She lived in Portugal until June 2008, when she and her family moved to The Hague, the Netherlands. After spending a year in a transitional class to learn Dutch, she enrolled in a program that combined two educational years in one and eventually graduated from Edith Stein College in 2013. She rediscovered her primary high school affinity with ethics during her bachelor degree in Liberal Arts & Sciences at Utrecht University, and soon decided to specialize in the ethics of technology. As a fulltime bachelor student, Ana co-wrote an interdisciplinary thesis on how Big History can contribute to the modernization of the Dutch education system, and wrote a disciplinary thesis on algorithmic personalization and its implications for personal autonomy. In her spare time, she had a part-time job in the HR department of PricewaterhouseCoopers (PwC) and enjoyed organizing extracurricular activities as a board member of the Giving Back Students Community (GBSC). She earned her bachelor's degree in 2017, and started her master's degree in Applied Ethics at the same university. She found her niche in biotechnologies and graduated a year later with a thesis on public trust in human embryo modeling but has delved deeper into the ethics as a PhD researcher at Maastricht University since August 2018. Alongside her research, Ana enjoyed tutoring Health Law and Health Ethics workshops to medical students, fulfilled the role of Junior Representative at her department, and became a board member of the Dutch Association for Bioethics. Currently, Ana is working as a post-doctoral researcher in the HipGametes project of the Pluripotent Stem cells for Inherited Diseases and Embryo Research (PSIDER) consortium, and as an ethicist in the Morphogenetic Micro Engineering team at the Institute for Technology-Inspired Regenerative Medicine (MERLN). She still lives happily in The Hague, with her fiancé, Dexter, and their two cats, Marley and Havanna.

Scientists are now able to bring together various types of pluripotent stem cells to cultivate cell structures that resemble human embryos at certain stages of early development. These so-called 'human embryo-like structures' could offer an (according to some, ethically and legally neutral) alternative to the use of human embryos in research. For example, because they can be produced on a large scale without requiring invasive procedures (such as egg donation) and because they are not subject to the laws and regulations that govern research with human embryos. In addition, the use of these structures allows certain elements to be added or removed, which enables unprecedented bottom-up approaches to the study of early human development. The goal of the research described in this book was therefore to determine whether and, if so, under what conditions scientific research with human embryo-like structures can indeed provide an ethically acceptable alternative to the scientific use of human embryos.