Research on Embryos and Embryonic Models for Scientific Use (EMSUs)

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Inserm Ethics Committee

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From research on embryos to research on embryonic models

As stated in our previous Memos, projects to improve knowledge of human embryos and develop embryo therapy techniques require research on human embryos. This research may be conducted on embryos conceived by in vitro fertilization (IVF) and no more needed for a parental project, but it can also use specifically constituted embryos or embryonic entities, as an increasing number of international scientific papers show[1]. Several French teams are participating in this movement, which led three researchers to contact the Inserm Ethics Committee (IEC) "Embryo and Developmental" Group, in order to share with it their ethical concerns and difficulties they sometimes encountered. Here is a brief presentation of their projects in order to illustrate the remarks and thoughts that will follow:

1. In order to study the consequences of supplying an exogenous mitochondrial genome that is not "selected" by the nucleus, followed by the coexistence of two different mitochondrial genomes within the same cell – in this case in a fertilized oocyte (zygote or first embryonic cell), Julie Steffann and Jean-Paul Bonnefont (Paris hospital group (APHP) Genetics – Imagine Inserm JRU1163), in collaboration with Nelly Achour Frydman (APHP Reproductive Biology), suggested analyzing the transcriptome of human embryonic entities obtained from unviable triploid zygotes, donated to research. The objective is to evaluate a potential harmful effect of the transfer of pronuclei. This project is essential in order to understand the possible consequences of mitochondrial replacement on cell physiology and is expected to supply major findings on the feasibility and safety of clinical trials already approved in some countries such as the UK, before it was discovered that incompatibilities between mitochondrial genome and nuclear genome could compromise development[2]. The French Biomedicine Agency (ABM) authorized the research project in May 2016. Later the Fondation Jérôme Lejeune (FJL) filed a court application for its annulment, maintaining that the intention was to clone human embryos and create transgenic ones. This request was rejected in June 2017 by the Montreuil Administrative Court, which FJL has since appealed.

2. Another French group, led by Laurent David (Center for Research in

1. In France, it is forbidden to create embryos for research. We will come back to this later on.
2. Ana Latorre-Pellicer et al., *Nature*, 2016, vol. 535, p. 561-565.

Transplantation and Immunology (CRTI), INSERM JRU1064), wishes to study the factors regulating cell pluripotency on human embryonic models constituted by the assembly of induced pluripotent stem cells (iPS) mimicking the cells of the pre-implantation epiblast and trophoblast stem cells. This research is conducted in collaboration with the team of Nicolas Rivron in Maastricht.

3. Finally, Pierre Savatier and his team (Stem Cell and Brain Research Institute (SBRI), JRU 1208) are participating in two international projects that involve the formation of chimeric embryonic entities. The first aims to determine whether the human iPS cells reprogrammed in the naive state of pluripotency are capable of colonizing the rabbit embryo, which would make it possible to evaluate their entire differentiation potential in all cell types constituting organs. The second seeks to study the capacity of human gene *ARGHAP11B* to increase neuron production. Since the effects of *ARGHAP11B* gene expression can only be optimally explored in the primate model, macaque transgenic embryos that express the human gene will be obtained from macaque oocytes. Then, following transfer of the embryos to the females, neuron production will be analyzed at different stages of macaque fetal development.

These various projects are part of a research field which has expanded recently and which has led to the creation of entities formed from cells or human elements recapitulating some aspects of embryonic development, but which are not embryos.

Several types of these artifacts have already been used and, as technical possibilities progress, many others are expected to appear in the near future. As John Aach and his colleagues emphasize: it will be possible to create artifacts that bypass canonical embryological stages, such as the appearance of the primitive streak[3]. As such, in 2014, Aryeh Warmflash and his colleagues created *gastruloids* from human embryonic stem cells[4], Yue Shao and his colleagues developed a model that simulates embryonic development after implantation and even after appearance of the primitive streak, from human stem cells[5], and the team of Magdalena

3. "With the growing power of synthetic biology to engineer complex tissues and tissue assemblies, it will soon become possible to generate SHEEFs that can bypass canonical embryonic stages through the use of completely different laboratory operations." (Addressing the Ethical Issues Raised by Synthetic Human Entities with Embryo-like Features, *eLife*, 2017, DOI : 10.7554/eLife.20674, p. 15)
4. Aryeh Warmflash & al., A method to recapitulate early embryonic spatial patterning in human embryonic stem cells, *Nature Methods*, 2014, vol. 11, p. 847–854.
5. Yue Shao & al., A Pluripotent Stem Cell-Based Model for Post-Implantation Human Amniotic Sac Development, *Nature Communications*, 2017, DOI : 10.1038/s41467-017-0023.

Zernicka-Goetz – albeit in mice and not humans – combined embryonic stem cells with trophoblast stem cells to form an entity with traits in common with the embryo, such as to enable the initial differentiation steps of several tissues and organs[6]. Nicolas Rivron and his colleagues for their part produced *blastoids* from stem cells, namely entities resembling blastocysts, also in mice[7].

To designate these artifacts, John Aach uses the expression "synthetic human entities with embryo-like features (SHEEFs[8])". We suggest the use of "embryonic models for scientific use" (EMSUs), which is a more intuitive expression that properly characterizes the type of entity in question (with phrasing along the lines of the term "animal model", for example)[9]. In this document, we will start by going over those EMSUs already used in research and those likely to be in the near future. Then we will examine how these entities differ from or are close to embryos, not just according to the biological point of view but also and, above all, to the ethical and legal perspective. Indeed, the question is knowing whether these embryonic models must be considered actual embryos and as such enjoy the same moral consideration, or be considered more as cellular constructions. Among others, Antonio Regalado asks: "Had they somehow made a real human embryo from stem cells? "[10]. It will also be necessary to think about the legal ban on creating embryos for research, creation that appears essential in order to answer certain fundamental questions – as show several recent studies conducted abroad on embryos created expressly for this purpose, either by *IVF* or by nuclear transfer (sometimes wrongly

6. Sally Ellys Harrison & al., Assembly of Embryonic and Extra-embryonic Stem Cells to Mimic Embryogenesis in Vitro, *Science*, 2017, 10.1126/science.aal1810. See also M. Shahbazi and M. Zernicka-Goetz, Deconstructing and Reconstructing the Mouse and Human Early Embryo, *Nature Cell Biology*, 2018, vol. 20, p. 878-887.
7. Nicolas Rivron & al., Blastocyst-like Structures Generated Solely From Stem Cells, *Nature*, 2018, vol. 557, p. 106-111.
8. Synthetic Human Entities with Embryo-like Features (SHEEF).
9. We also encounter the expressions "embryoid", "artificial human embryo", "virtual human embryo", (Antonio Regalado, Artificial Human Embryos Are Coming, and No One Knows How to Handle Them, 19 septembre 2017, accessible à https ://www.technologyreview), "embryon synthétique" (M. Shahbazi et M. Zernicka-Goetz, *art. cit*., p. 884), "corps embryoïde" (Martin Pera & al., What If Stem Cells Turn into Embryos in a Dish?, *Nature Methods*, vol. 12/10, 2015, p. 917), "structures de type embryon" (CCNE, avis 129, 2018, p. 11), as well as "construction biotechnologique" (Benjamin Hurlbutt & al., Building Capacity for a Global Genome Editing Observatory : Conceptual Challenges, *Trends in Biotechnology*, vol. 36/7, 2018, p. 639). This plethora of designations highlights the difficulty of finding one that covers all the entities concerned and denotes the conventional and as such imperfect aspect of these expressions. In our discussions at the IEC, other names had been envisaged, such as *Artefacts embryoïdes [embryoid artifacts]*, *Modèles expérimentaux de développement embryonnaire* [experimental models of embryonic development] or *Modèles embryonnaires humains de synthèse* [synthetic human embryonic models].
10. Antonio Regalado, *art. cit*. The use of stem cells is however also subject to ethical and legal requirements.

referred to as “cloning”[11]). In addition, the latest statement (129) of the French National Consultative Ethics Committee (CCNE) invites thoughts on the possible exceptions to this ban[12].

Categories of embryonic models for scientific use in research

When studying the properties of embryos, several types of entities are likely to be used:

1. The various *EMSUs.* They can be created because the stem cells are capable of forming structures that recapitulate aspects of embryo organization and development[13]. Some authors such as John Aach or Antonio Regalado refer to the emergence of “synthetic embryology”.

2. The *chimeras*. These are organisms that contain at least two groups of genetically different cells, coming from individuals or different species (intraspecies or interspecies chimeras). These are obtained by introducing pluripotent stem cells, embryonic stem cells (ESC) or iPS cells into an embryo (blastocyst). Each cell population retains its own genetic character and the result is a mosaic. The interspecies chimeras notably include human-animal chimeras (human embryo into which animal cells are introduced) and animal-human chimeras (animal embryo comprising human cells).

3. The *hybrids* and *cybrids*. A hybrid is formed when a spermatozoa from one individual is used to fertilize the ovum from another individual of a different species. As a consequence, each cell of the hybrid organism has the chromosomes of both species. A cybrid is a cytoplasmic hybrid created when the nucleus of a cell of an organism is introduced into an enucleated ovum of an individual from another species or the same species. The cybrid is a virtual-clone of the organism whose nucleus has been transferred[14]. Hybrids and cybrids are often, inaccurately, referred to as chimeras. We must emphasize again that many countries ban the creation of

11. Cloning is a procedure used to identically reproduce the initial biological entity, for example monoclonal antibodies, which are all identical to each other. Nuclear transfer can be a technical stage of cloning if the purpose is reproductive and the transfer is between two syngeneic animal cells (with the same genome). This is not the case for scientific research in which the embryo must be destroyed at the end of the experiment and in which the embryonic entity constructed is not similar to the embryo supplying the nucleus transferred.
12. CCNE, *Contribution du CCNE à la révision de la loi de bioéthique*, avis 129, 2018, p. 58.
13. Martin Pera & al., *art. cit*., p. 917. See also David Turner & al., Organoids and the Genetically Encoded Self-Assembly of Embryonic Stem Cells, *Bioessays*, vol. 38, 2015, p. 181-191.
14. Human Fertilisation Embryology Authority, *Hybrids and Chimeras*, octobre 2007.

entities combining human and animal genetic heritage.

4. The *parthenotes*. These are embryos obtained through parthenogenesis, i.e. through the division of an unfertilized female gamete[15].

5. The embryos constituted by the micromanipulation of constituent cell elements (e.g. mitochondrial donation) or by eliminating some of their constituents (e.g. restoration of diploidy). Mitochondrial replacement therapy is a case of intraspecies cybrid.

6. The embryos created for research by *IVF* as it has been done in three recent publications involving the use of CRISPR-Cas9[16] and in a fourth, conducted by Puping Liang et al., the embryos were produced by nuclear transfer[17].

The ethical issue raised here is that of knowing how these entities must be considered – whether they have a moral status and, if so, what status, especially if it is not necessarily the same in all cases envisaged. Antonio Regalado asks: "What's really growing in the dish? There is no easy answer to that."[18]. It is immediately evident that one especially important point in answering this question concerns the future of these entities: if implanted, could they develop just like embryos and as such have a similar moral status to that of human embryos? For some of them, it is clearly not the case – particularly for those lacking the structures necessary to generate a placenta, heart or brain[19]. This category includes the various embryonic models mentioned at the beginning of this document, like the gastruloids. We are therefore talking about cellular constructions. Other EMSUs develop a primitive streak in the form of a ring[20]: such artifacts also do not have characteristics similar to those of embryos. The researchers that produce these models also carefully and intentionally avoid constructing entities that could develop just like embryos following

15. "Parthenotic embryos ('parthenotes') and 3PN embryos can and have been used as alternatives for 'normal' embryos in research, including basic research on germline gene editing" (Guido de Wert & al., Responsible Innovation in Human Germline Gene Editing, *European Journal of Human Genetics*, 2018, p. 8, https ://doi.org/10.1038/s41431-017-0077-z). It should be noted that the European Court includes clones and parthenotes in the category of embryos.
16. Hong Ma & al., Correction of a pathogenic gene mutation in human embryos, *Nature*, vol. 548, 2017, p. 413-419, Yanting Zeng & al., Correction of the Marfan Syndrome Pathogenic FBN1 Mutation by Base Editing in Human Cells and Heterozygous Embryos, *Molecular Therapy*, vol. 26/11, novembre 2018 and Lichun Tang & al. CRISPR/Cas9-mediated gene editing in human zygotes using Cas9 protein, *Mol Genet Genomics* 2017.
17. Puping Liang & al., Correction of ß-thalassemia Mutant by Base Editor in Human Embryo, *Protein Cell*, on-line, 2017 : "To model homozygous mutation disease embryos, we constructed nuclear transfer embryos by fusing the lymphocyte or skin fibroblast cells with enucleated in vitro matured (IVM) oocytes."
18. Antonio Regalado, *art. cit.*
19. Antonio Regalado, *art. cit.*
20. Martin Pera & al., *art. cit*., p. 918.

potential implantation, precisely in order to avoid ethical issues[21].

But are there embryonic models that could develop like an embryo? This is not the case of the chimeric embryos, such as those used by the team of Ali Brivanlou, in which human stem cells were transplanted into a chicken embryo[22], or those produced by Pierre Savatier, by inserting human iPS cells into rabbit embryos[23]. In these two cases, it would be more judicious to refer to xenografts rather than chimeras. In addition, the purpose of these models is to study the behavior of human cells during their early development and not to produce an animal with human cells. The situation differs slightly for chimeras created from interspecies blastocysts for the purpose of producing tissues and organs for potential transplants: research was conducted in animals, mainly pigs, in order to grow human organs, such as a pancreas[24]. In this case it will involve implanting a chimeric embryo in an animal uterus, and to allow development to continue well after its birth. It is understandable then, that Usha Lee McFarling stresses that these chimeras must be human enough to serve as effective models for research and even in therapeutic research, if possible, but not so human that they qualify for the human beings' protection altogether[25]. All that remains is to know how to determine this[26]. Another EMSU with potential for development following possible implantation is an embryo created from gametes derived from stem cells or their precursors – moreover, in this case, an embryo will have been created by bringing a spermatozoon and an ovum together[27].

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21. Insoo Hyun, Engineering Ethics and Self-Organizing Models of Human Development : Opportunities and Challenges, *Cell*, vol. 21, 2017, p. 719. The same author states later on that another way of achieving this objective would be to genetically modify an embryo by inactivating the CDX2 gene; however, the problem is that this procedure could be described as the creation of a handicapped embryo (p. 720).
22. Ian Martyn & al., Self-organization of a Human Organizer by Combined Wnt and Nodal Signalling, *Nature*, 23 mai 2018.
23. While French law forbids the creation of chimeras, this only concerns human-animal chimeras, in which animal cells are incorporated into a human embryo.
24. Tomoyuki Yamaguchi & al., Interspecies Organogenesis Generates Autologous Functional Islets , *Nature*, 2017, doi :10.1038/nature21070, et Jun Wu & al., Interspecies Chimerism with Mammalian Pluripotent Stem Cells, *Cell*, 2017, vol. 168, p. 473-486.
25. “It raises] serious ethical dilemmas about the moral status of these part-human animals. Chimera test subjects must be human enough to serve as effective models for health research, but not so human that they qualify for protection from this research altogether.” (Near the Campus Cow Pasture, a Scientist Works to Grow Human Organs – in Pigs, *Stat*, 20 octobre 2017, https ://www.statnews.com/2017/10/20/human-pig-chimera/?utm_source=STAT+Newsletters&utm_campaign=2f8e620631-MR&utm_medium=email&utm_term=0_8cab1d7961-2f8e620631-149620841)
26. Nita Farahany & Henry Greely, The Ethics of Experimenting with Human Brain Tissue, *Nature*, 2018, vol, 556, p. 429-432.
27. Naoko Irie & al., SOX17 Is a Critical Specifier of Human Primordial Germ Cell Fate, *Cell*, 2015 vol. 160, p. 253-268.

Artifacts devoid of this capacity for development – such as the cellular constructions based on stem cells – should be exempt from the legal and moral barriers applicable to research on human embryos[28]. For example, as John Aach and his colleagues report, the rule applicable in the English-speaking countries – and many others besides –, stipulating that embryo studies can only take place within 14 days after fertilization or before the appearance of the primitive streak, could become obsolete with research using such entities[29]. In France the time limit recommended by the Comité Consultatif National d'Ethique (CCNE) is 7 days; this marks time of implantation. The CCNE also emphasizes that "theoretically, such research could be carried out as long as the *in vitro* embryo's development is (or will be in future) technically possible. There is nothing in the law as it is currently written to prohibit this from taking place"[30], and it can be hoped that the legislator will address the issue. Irrespective of the legislator's response, this limit should not apply to EMSUs that do not have the capacity to develop with a view to being born. Moreover, the French ban on creating embryos for research purposes will not apply, insofar as these entities differ by their absence of late development potential.

It must be noted that chimeras and cybrids raise yet another issue, that of specific identity and integrity: do such entities belong to the human species? This depends on the level of chimerism[31] as well as their proximity to what constitutes essential human characteristics, particularly if they contain neurons or a germ-line cell of human origin. To this must be added the psychological importance of external appearance, with those beings that resemble us being more easily considered human[32], hence the relevance, for example, of inhibiting any gene enabling ossification of the humanoid facial skeleton. The existence of these chimeras does, however, blur the separation of the species and could consequently represent a threat to the identity and integrity of our humanity. A concern that differs from that of the moral status discussed in this document, but for some it constitutes an argument

28. These entities "offer a possible way of escaping the [ethical] dilemmas by enabling generation of human entities that recapitulate aspects of embryonic development potentially very precisely, but that are different enough from non-synthetic embryos to justify their exemption from research limits on such embryos." (John Aach & al., *art. cit.,* p. 3)
29. Helen Shen, Embryo Assembly 101, *Nature*, 2018, vol. 559, p. 20.
30. CCNE, avis 112, 2010, p. 51.
31. Currently, for human-animal chimeras, the proportion of animal cells is 1% (human/pig) and 0.01% (human/sheep) (Jun Wu & al., *art. cit.*).
32. In regard to EMSUs, Yue Shao emphasizes that his model does not have a human form (*art. cit*., p. 11).

in favor of not creating such chimeras[33].

The issue of moral status

In order to know the ethical requirements that apply when using EMSUs, we need to determine their moral status and compare it to that of embryos, which is used here as a reference criterion in order to see whether the requirements are the same in both cases[34]. Indeed, there is the risk of these entities possessing an unclear and even ambiguous legal and moral status, as underlined by Insoo Hyun[35].

Above all, let us ask what it means to have a moral status. The idea of moral status is characterized by Mary Anne Warren: "To have moral status is to be morally considerable, or to have moral standing. It is to be an entity towards which moral agents have, or can have, moral obligations"[36]. There are beings that count morally and towards which we have obligations, others that do not. The beings that count morally are often called "moral patients" and those that have obligations are "moral agents". An adult human being is both; a neonate is solely a moral patient. The category of moral patients differs among authors. For some, only human beings count morally (this is the anthropocentric position). For others, all beings which can feel suffering or pleasure are moral patients: they have interests that must be taken into account (this is the pathocentric position). While this primarily concerns animals, it would also apply to human embryos and EMSUs, were they to possess some sentience[37].

How is the moral status of a being determined? We have just seen that it is based on some of its *characteristics* or *aspects*[38], such as "being human" or "having sentience". But more specifically?

33. Dietmar Hübner, Human-Animal Chimeras and Hybrids : An Ethical Paradox behind Moral Confusion?, *Journal of Medicine and Philosophy*, 2018, vol. 43, p. 187-210. Accepting this argument implies that we ascribe a moral weight to belonging to the species and as such that we forsake moral individualism, the dominant position in our ethical and legal tradition. See also i Françoise Baylis, Animal Eggs for Stem Cell Research : A Path Not Worth Taking, *AJOB*, 2008, vol. 8/12, p. 18-32.
34. This is at least how the question is usually asked. There are, however, particularistic or casuistic approaches that consider the concept of moral status to be irrelevant here. Furthermore, some ethical requirements can be based on considerations other than that of moral status, such as symbolic values – which will not be discussed in this document.
35. Benjamin Hurlbut & al., Revisiting the Warnock Rule, *Nature Biotechnology*, vol. 35/11, 2017, p. 1034.
36. Mary Anne Warren, *Moral Status*, Oxford, OUP, 1997, p. 3.
37. Bernard Baertschi, *Enquête philosophique sur la dignité*, Genève, Labor et Fides, 2005, p. 181-195.
38. The philosophers refer to "properties". To avoid any ambiguity, we will use the term "characteristics".

All beings possess two types of characteristics, intrinsic (internal) and extrinsic, which can be defined as follows:

[D1] *Intrinsic* is when it characterizes the being itself, independently of what surrounds it.

[D2] *Extrinsic* is when it characterizes the being according to what surrounds it.

The two most important types of extrinsic characteristics are *relational* properties and *instrumental* characteristics. For instance, the utility that a farm animal offers its owner is an instrumental characteristic of that animal, whereas a mother's love for her child is a relational characteristic of the child (it is loved). Self-awareness or sentience are however, intrinsic characteristics which a human being continues to possess, even if he is unloved and abandoned on a desert island. As we see, these characteristics sometimes carry values: the farm animal has a utility value for its owner, the child has relational (emotional) value for its mother, self-awareness an intrinsic value for human beings. As such, we would say:

[D3] The intrinsic (or extrinsic) value of a being is the value possessed by this being or that which we attribute to it[39] in virtue of its intrinsic (or extrinsic) characteristics[40].

Regarding human beings, the characteristics which are a source of intrinsic value and which count in order to determine its moral status are traditionally those related to rationality. For Kant for example, these are self-awareness and autonomy. The *Universal Declaration of Human Rights* of 1948 says the same: "All human beings are born free and equal in dignity and rights. They are endowed with reason and conscience" (Art. 1). If reason plays this role, it is that it denotes the essential characteristic that human beings attribute to themselves and that separates them from animals. Leon Kass refers to the *human core* that makes us "more than beasts yet less than gods"[41], and Chloé Giquel stresses that "the distinction between humans and animals is based mainly on self-awareness"[42] – a form of self-awareness that implies rationality –, with Sonia Desmoulin-Canselier commenting

39. If we say "the value possessed by this being or that attributed to it", it is to avoid taking a stand on the question of knowing whether the values are objective or subjective.
40. Wlodek Rabinowicz et Toni Rønnow-Rasmussen, A Distinction in Value : Intrinsic and for its Own Sake, *Proceedings of the Aristotelian Society*, 2000, p. 34-35. Not all characteristics are of course sources of value, at least not value that counts for moral status: having blue eyes is an intrinsic characteristic without moral value, and many relations are in the same case (think of spatial relations, such as "being to the right of").
41. Reflections on Public Bioethics : A View from the Trenches, *Kennedy Institute of Ethics Journal*, 2005, vol. 15/3, p. 240.
42. La création d'animaux chimères porteurs d'organes humains, *Médecine et droit*, 2016, p. 46

that “for many people, humanity is defined in opposition to the animal kingdom, likened to bestiality”[43].

Linking intrinsic value to *dignity* is also a permanent feature of our tradition. Again for Kant, a representative of the anthropocentric approach, an animal – i.e. a thing –, has no intrinsic value, only instrumental value to its owner: such a being has “merely a relative worth, i.e., a price” to the contrary of people who have “an inner worth, i.e., dignity”[44], which forbids all instrumentalization. These days, the CCNE uses the same expressions: the respect of dignity “demands that people should never be considered simply as means towards an end, but an end in themselves, and should never be instrumentalized” [45]

The moral status of potential person

What about the moral status of the embryo, at present? An embryo has no intrinsic characteristics denoting rationality or – at least in the beginning – no sentience, because it has no nervous system[46]. But it will possess them if it is implanted and develops normally. It therefore possesses them *potentially* and must as a consequence be considered a potential human person according to the CCNE. However, by that the CCNE emphasizes in its statementno. 106 that it does not intend to define the nature of the embryo, but refers to the law that considers that a human being is a person only at birth: if a human being becomes a person at birth, as states the law, then it is a potential person before that point.

It is nevertheless also possible to argue in favor of this theory from the ethical and philosophical points of view; as such, Normal Ford defines the human embryo as “A totipotent single-cell, group of contiguous cells, or a multicellular organism which has the *inherent* actual potential to continue species specific i.e. typical,

43. Une chimère homme-animal comme modèle expérimental pour développer des vaccins contre les zoonoses ?, in Christian Byk (dir.), *Manuel francophone d’études de cas cliniques en bioéthique*, Paris, MA éditions, 2016, p. 241.
44. Imanuel Kant, *Groundwork for the metaphysics of morals*. Already in the 13e century, Aquinas said the same : ‘Dignity means the goodness something possesses because of itself, utility its goodness because of another’ (*Scriptum super sententiis,* liv. 3, d. 35, q. 1, a. 4, q. 1, c, www.corpusthomisticum.org/iopera.html), and he spoke of ‘the dignity of manhood, in so far as he is naturally free, and exists for himself’ (*Summa Theologica,* IIa IIae, q. 64, a. 2, ad 3).
45. CCNE, *Questionnement pour les États généraux de la bioéthique*, avis 105, 2008, p. 106.
46. Pera and his colleagues note that one of the reasons for banning testing on embryos beyond 14 days in the UK is that this point “also marks the beginnings of the central nervous system” (*Art. cit.,* p. 918).

human development"[47]. More formally, we would say that:

[D4] An entity is a *potential person* if and only if it has the capacity to develop into a person in the hypothesis of an implantation.

The moral status of the embryo is therefore determined by the characteristics which confer on it its intrinsic value and which are potentialities, regardless of the environment in which it is currently placed. As such, a transferred embryo and a frozen embryo are both potential people. If they merit a form of respect it is because they are potential people, in that they have in them what is necessary to subsequently acquire – if they are implanted and develop – the rational characteristics inherent to what makes a living being a person.

As a consequence, determining the moral status of EMSUs involves asking whether these artifacts are – like the embryos themselves – potential human people. This underlines that, ethically, what counts here is not the *method* of producing these beings, but the characteristics that define them (the *product*). As we shall see, some legal approaches also consider that were an embryonic entity to be created by a method other than fertilization, this would not be enough to deprive it of the name and status of embryo. On this point, these legal approaches and the ethical approach that we have adopted tend to be in agreement: an embryo is a potential human person and the embryonic models will be too if they should have the potential to develop into people.

This confirms that there is no single or easy answer to the question of knowing whether a particular EMSU has the potential that would make it a potential human person and as a consequence give it the same moral status as an embryo[48]. The response to this question is not conceptual, but biological and empirical; it can therefore only be given in accordance with the knowledge of the biological and genetic characteristics of this artifact. The ultimate proof would be to implant it and observe the result – which would nevertheless be morally wrong and forbidden by law.

47. Cited in Catherine Stanton and John Harris, The Moral Status of the Embryo Post-Dolly, *Journal of Medical Ethics*, vol. 31, 2005, p. 223. Emphasis added.
48. It also cannot be asserted that EMSUs would be potential embryos, except in the trivial sense in which, via reprogramming, it could be said of any pluripotent cell or any somatic cell whose totipotency is restored (Bernard Baertschi et Alex Mauron, Moral Status Revisited : The Challenge of Reversed Potency, *Bioethics*, 2010, vol. 24/2, p. 96-103).

The legal status of the embryo

We have just discussed how the question of embryo status can be understood on the ethical level. Does the law take the same approach? Not exactly. The law is primarily interested in two questions: what an embryo is and how to ascertain the protection to confer on it. The first question is important in that there are laws concerning the embryo, and so it is important to know whether the models and other embryonic constructions are concerned by them.

French law proposes no positive or explicit definition of the embryo. However – and this concerns our question quite directly – the judge already made exclusions from the concept of the embryo, ruling that some entities were not embryos; as such, this concerns embryonic stem cells which because of this evade provisions regulating testing on human embryos[49]. However, it must be emphasized that, in the aforementioned judgment, the administrative judge did not consider the case of *combinations* of stem cells and certainly not of iPS cells – in short, EMSUs. Given the rate at which medical and scientific research is progressing, it is tricky to define the scope of this decision.

The Court of Justice of the European Union (CJEU) was less timorous when it came to the positive definition of the embryo. On October 18, 2011, in the *Brüstle* judgment[50], the main subject of which was the patentability of a procedure using stem cell sampling with regard to Directive 98/44/EC relating to the legal protection of biotechnological inventions, the Court gave an autonomous definition of the embryo, imposed on the Member States: “Any human ovum after fertilization, any non-fertilized human ovum into which the cell nucleus from a mature human cell has been transplanted and any non-fertilized human ovum whose division and further development have been stimulated by parthenogenesis constitute a ‘human embryo’ within the meaning of Article 6(2)(c) of the Directive” and adds that “it is for the referring court to ascertain, in the light of scientific developments, whether a stem cell obtained from a human embryo at the blastocyst stage constitutes a ‘human embryo’ within the meaning of Article 6(2)(c) of the Directive.”

It is interesting to note that this Court considers that what constitutes an embryo

49. Paris Administrative Court, January 21, 2003, no. 0207626/6, Alliance pour les droits de la vie (Alliance for the Rights to Life): “Considering that under the terms of Article L.2141-7 of the French Public Health Code, a human embryo cannot be conceived or used for commercial or industrial purposes; that stem cells cannot be regarded as embryos […]”.
50. Court of Justice of the European Union, October 4, 2011, *Brüstle*, C-34/10.

is either a process (fertilization, parthenogenesis), or a result (a cell possessing a mature human nucleus). This dual approach is found in various legislations. While in general the basic definition of the embryo is that of the product of the union of a sperm and an egg – in short, fertilization – many countries leave the door open to subsequent specifications. In Germany, an embryo is characterized by its potential for development, as in the Netherlands and Belgium where an embryo is a collection of cells with the capacity to develop into a human being and in Australia, where any embryonic entity with the capacity to develop beyond the primitive streak stage counts as an embryo[51]. In all cases, what characterizes the embryo is therefore not so much fertilization but the potential for development. In addition, the CJEU says the same in the *Brüstle* judgment because it refers for the embryo to an "inherent capacity of developing into a human being", which comes back to potentiality based on intrinsic characteristics[52].

What about the protection currently conferred on embryos by law? Here, French law takes a path other than ethics. To determine it, it does not refer to a status based on intrinsic characteristics, but on the situation in which the embryo is found. As Laurence Brunet and Sonia Desmoulin-Canselier state, it is not the ontological nature of the embryo that is important in law, but its *teleology* – the purpose it will serve[53]. They refer to the embryo *in vitro*, i.e. exactly the type of embryo that concerns us here and assert that what confers or does not confer protection on it is the existence of a parental project, namely its purpose[54]. Claire Neirinck shares this view, stating that it is not freezing that marks the cut-off between the embryo-human and the embryo-thing; it is the end of the parental project.[55] Although freezing places the embryo outside of time and therefore suspends its fate, while the parental project

51. Martin Pera & al., *art. cit.*, p. 919.
52. For the use of the terms "capacity" and "potentiality", refer to Bernard Baertschi, Jean-François Guérin and Pierre Jouannet, *Regards sur l'embryon*, Paris, Le muscadier, 2019, part 3.
53. Human Embryo, Animal Embryo, Chimerical Embryo : What Legal Status in French Law?, *Journal of Civil Law Studies*, 2008, vol. 1, p. 90 : "We are going to see how the status of *in vitro* embryo obliges to set aside all ontological definitions of the embryo to restrict its to a teleological definition : what is important is the use intended for the in vitro embryo". The CCNE had also noted that legislators "considered that not being able to resolve the question of the embryo's nature did not, in practical terms, prevent a ruling on how it should be treated. With subtlety, legislators preferred to focus on the embryo's potential future rather than on its present existence." (Opinion 105, p. 6)
54. « In vitro embryos have a very ambiguous legal status : on the one hand, they benefit from full legal protection when they are part of a parental project. On the other hand, as soon as there is no such project (e.g. no married couple is willing and able to receive the *in vitro* embryo available for adoptive implantation), they count for nothing. » (*Art. cit.,* p. 91)
55. L'embryon congelé sous le regard d'un juriste : au-delà de la qualification, *Journal international de bioéthique*, 2017, vol. 28/4, p. 123.

continues to persist, the embryo preserves a value making it much more than an object or a collection of human cells.

At first glance, the legal approach appears to differ greatly from the ethical approach that we followed, because the former bases its considerations on relational characteristics (the parental project and therefore the utility of the embryo for that project), whereas the latter is based on intrinsic characteristics (ontology: what the embryo *is* in itself). However, and this point is crucial to our discussion, the opposition fades away as soon as we become aware of a second important ethical factor, the *future* of the embryo. Indeed, as the CCNE stresses, what counts on the ethical level is not primarily that the embryo is a potential human person but that it is an *evolving* potential human person. The parental project is precisely an important factor in determining this fate, and it is clear that the EMSUs are totally foreign to such a project[56].

The moral status of the evolving potential person

The adjective used when referring to "potential person" is sometimes a little ambiguous. Indeed, we might think that a spare embryo donated to research is not a potential person because it will not be able to become one, given that it will not be implanted. However, the intrinsic characteristics approach does not see things that way, given that such an embryo, if implanted, could develop into a person. It is therefore well and truly a potential person. However, contrary to an implanted embryo, it is not an *evolving* potential person. Distinguishing potential people from evolving potential people is fundamental here.

As such, in the case of the embryo, certain relational characteristics count, given that it constitutionally depends on a parental project for the achievement of its potential. Given that both intrinsic *and* extrinsic conditions are as a consequence necessary for the protection of potential human people, EMSUs cannot benefit from the protection reserved for evolving potential people, like human embryos without a parental project. Indeed, as emphasizes Pierre Jouannet, "when a child is born through *in vitro* fertilization, the reason for being, situation and future if not the status of the embryos, conceived during the same attempt and cryopreserved since then,

56. CCNE, Ethical reflection concerning research on human embryonic cells and on human embryos in vitro, October 21, 2010 (opinion 112), p. 6: "It is because of the existence and persistence of this human bond that an embryo in vitro, already a 'potential human being', becomes an incipient 'potential human being'".

will inevitably change"[57], because they are liable to become spare embryos, thereby losing the possibility of becoming people. While they formally remain potential human people, they are now deprived of a future unless donated to another couple, in which case they become part of a second parental project.

The parental project is a subjective relational characteristic (it is rooted in the minds of the parents); but there is another objective relational characteristic that counts: implantation in the uterus. This characteristic is certainly linked to the parental project because it is the parents who decide whether or not to transfer the embryos in the uterus to implant, and so to the realization of the potentiality of the person of the embryo, it is a necessary causal condition: without implantation, we cannot refer to an evolving potential person[58]. It follows that no *in vitro* embryos or EMSUs objectively possess in the same making potential person status (even if some embryos possess it subjectively because of the parental project). In addition, it must be noted that any embryo not implanted within 7 days of fertilization loses all capacity for development *in utero*, and is therefore no longer a potential human person – as is the case of EMSUs that are cellular constructions devoid of all capacity for development.

We arrive at the same conclusion if we consider research. As we know, three types of research exist: fundamental, preclinical and clinical. The embryos used in the first two types of research will never be implanted and have never been or no longer are the subject of a parental project; they are therefore not evolving potential people. Certainly they could have been potential people, at least some of them, those which had they been implanted would have developed until birth, but this is not what is most important. It should never be forgotten that ascribing potential human person status to embryos or to other entities does not constitute a specific obstacle to research involving them, because research on human embryos is lawful.

Consequently, what counts on the ethical level is knowing whether the embryo is a potential human person, certainly – embryos exist that have no such potential, which are not transferable –, but also and above all if it is an *evolving* potential person. The cessation of the parental project or the foregoing of any implantation are decisions that destroy this future. The research could then be morally justified if the

57. Pierre Jouannet, L'embryon sujet : patient d'une médecine de l'embryon ? L'embryon objet : quelle recherche et pour qui ?, in Pierre Jouannet et Catherine Paley-Vincent, *L'embryon, le fœtus et l'enfant*, Paris, Eska, 2009, p. 81.
58. The project to construct artificial uteruses will not be discussed in this document.

embryo on which it is performed is no longer or never will be an evolving potential person – and if all the other conditions for its lawfulness are fulfilled. Same for the EMSUs, which are never evolving potential people but at the most potential people (if they have a development potential analogous to that of embryos), and for the majority simple cellular constructions.

In short, if EMSUs are not potential human people, they could be used in research like cell collections or human tissues. If they are potential human people then they could be used for research like embryos are, in accordance with the conditions laid down in law. There is consequently a certain ethical parity among embryos, EMSUs and analogous entities, which raises a new question: would it not be judicious to reconsider the ban on creating embryos for research?

The creation of embryos for research

In its statement no. 112, the CCNE emphasizes that creating human embryos for research purposes is unacceptable because it would be done independently of any enrolment in the human line, i.e. any parental project, whether or not it is likely to be abandoned. However, in its latest statement (no. 129), the same CCNE envisages the possibility of exceptions, justified by medical purposes. What should we think of this?

The creation of embryos for research is forbidden in France, as it is in all countries having ratified the Oviedo Convention[59]. However, the question has recently become the subject of discussion and we have mentioned that several studies have used embryos explicitly produced for research. Indeed, some studies have no other possibility than to be performed on embryos created for this reason. One example is research on embryos with a specific genetic mutation, in order to try to find a way of correcting it at the zygotic stage. These embryos could be created from the gametes of people carrying the mutation, donated to research.

Currently, this type of research can be carried out on embryos carrying abnormalities known to severely disrupt their development and which are therefore neither transferred to the uterus nor frozen. This is the case, for example, of the triploid zygotes, which possess an additional pronucleus, multifragmented embryos or those with genetic or chromosome abnormalities detected by pre-implantation

59. “The constitution of human embryos for research purposes is forbidden” (Art. 18.2).

genetic diagnosis (PGD). In the latter case, such research can only be done following the genetic diagnosis, namely on the 4th or 5th day of development, a stage much too late to envisage effective genetic correction of all the cells of the embryo. Recently a Chinese team created Marfan syndrome carrier embryos which were treated apparently with a certain level of success, it would appear, by a method known as base editing[60].

Creating embryos for research would also make it possible to study the embryo in the very first days of its existence, in order to improve assisted reproductive technology (ART) techniques – this is one of the exceptions envisaged by the CCNE in its statement no. 129. Indeed, in order to study the cellular and molecular mechanisms regulating early development, it is necessary to have embryos that are in principle normal. The embryos that are frozen after IVF and which are no longer needed for the parental project of their genitors can be donated to science for this purpose. However, in the majority of cases, the embryos are frozen at the 4/8 cell or blastocyst stage, thereby making it impossible to study events occurring beforehand.

If we focus on the history of ART, we realize that the creation of embryos to improve and even develop *in vitro* fertilization (IVF) techniques has already taken place. These days, IVF is a recognized medical procedure routinely carried out all over the world. However, Louise Brown would never have been born in 1978 if Robert Edwards, Patrick Steptoe and their colleagues had not persevered with their research despite the hostility they encountered at the time[61]. The first results of human IVF with the creation of embryos were published in 1969[62]. Two years later, the same team described the culture of human embryos up to blastocyst stage[63]. Attempts were made for 10 years up to Louise Brown's birth in 1978[64]. Apart from the laboratory experiments, we know that between 1969 and 1978 this team had undertaken 457 cycles of IVF treatment for 250 women which had led to 112 embryo transfers, 5 clinical pregnancies and 2 births[65]. It can be concluded that many

60. Yanting Zeng & al., *art. cit.* The same approach is found in the United States ; cf. Hong Ma & al., *art. cit.*
61. Simon Fishel, First in vitro fertilization baby – this is how it happened, *Fertility and Sterility*, 2018, vol. 110/1, p. 5-11.
62. Robert Edwards & al., Early stages of fertilization in vitro of human oocytes matured in vitro, *Nature*, 1969, vol. 221/5181, p. 632-635.
63. Patrick Steptoe & al., Human blastocysts grown in culture, *Nature*, 1971, vol. 229/5280, p. 132-133.
64. Patrick Steptoe & al., Birth after the reimplantation of a human embryo, *Lancet*, 1978, vol. 2/8085, p. 366.
65. Simon Fishel, *art. cit.*

embryos had been created for research and that many attempts similar to clinical research, had been undertaken in other countries before IVF became a reality and the legislator banned the creation of embryos in France.

Sometimes also, we realize retrospectively that conducting research on embryos would have been ethically necessary, as shows the case of IVF using round spermatid injection (ROSI).

When a man has no sperm in his semen (azoospermia), intra cytoplasmic sperm injection (ICSI) can be attempted using spermatozoa surgically taken from the testicles. ICSI is mandatory because, having not undergone epididymal maturation, the spermatozoa cannot fertilize the oocyte. If obstructive azoospermia is involved, spermatogenesis is in general normal and many spermatozoa can be collected from the testicles without problem. However, non-obstructive azoospermia is due to a major spermatogenesis defect and very few spermatozoa, or none at all, are produced. Nevertheless, careful dissection of the testicular tissue can sometimes obtain a sufficient number of spermatozoa to perform an ICSI of the various oocytes collected from the woman. If no spermatozoa can be collected, it was proposed to microinject, when possible, a spermatid which is the precursor cell of the spermatozoon. Derived from meiosis, the spermatid is a haploid cell that differentiates into a spermatozoon through a process called spermiogenesis during a period of over 15 days. At the end of spermiogenesis, the spermatid elongates and its constitution is very close to that of the spermatozoon. However, at the beginning of spermiogenesis, the spermatid is round and its constitution is very different. Several teams had attempted the clinical use of round spermatids, with a French team reporting the birth of a child in 1995[66] – which received a lot of media attention at the time.

Attempting to bring a child into the world following round spermatid ICSI was extremely audacious and borderline careless. Indeed, while the chromosomal and genetic content of the round spermatid is in principle identical to that of the spermatozoon, the nuclear proteins surrounding the DNA are totally modified during spermiogenesis. These protein changes are not without influence on the decondensation of the male pronucleus and the epigenetic modifications occurring at its level within the zygote following fertilization. In addition, the spermatozoon

66. Jan Tesarik & al., Viable embryos from injection of round spermatids into oocytes, *New England Journal of Medicine,* 1995, vol. 333/8, p. 525.

supplies cytoplasmic elements important for embryo formation – on the one hand, a cytosolic factor that activates the oocyte by triggering calcium pulses, on the other, the centrosome that organizes the first cell mitoses. It is also this immaturity of the paternal cell used to form the embryo which can explain the low rates of fertilization, implantation and birth obtained when round spermatids were used in ICSI[67]. It is therefore not surprising that the American Society for Reproductive Medicine (ASRM) had published in 2008 a statement recommending that the technique be considered experimental, given the many unresolved questions and the uncertainties surrounding the health of the children[68]. Concern which has since intensified when DNA methylation abnormalities of the male pronucleus were shown in mice when the embryo was formed from a round spermatid[69].

In fact, very few teams have pursued this type of ICSI, except in Japan. Recently, the Japanese team which doubtlessly has the larger experience in the matter published an article reporting the follow-up of 90 children from birth to 2 years of age[70]. While no major abnormalities had been observed at that age, there is no guarantee that abnormalities consecutive to possible epigenetic disruptions in the early embryonic stages will not appear later during the development of the children. We can wonder whether the risk of premature vascular aging leading to hypertension, which was detected in adolescents conceived by IVF[71], would not be higher in children conceived with round-spermatid ICSI.

This retrospective analysis clearly shows that it would have been justified to undertake studies on embryos obtained through round-spermatid ICSI before considering their transfer for gestation.

As shown by the various situations presented above, the scientific and medical purposes concerned, some of which emphasized by the CCNE, would justify lifting the ban on creating embryos for research, even though these embryos are created

67. Irfan Aslam & al., Can we justify spermatid microinjection for severe male factor infertility? *Human Reproduction*, Update 1998, vol. 4/3, p. 213-222.
68. The Practice Committee of the American Society for Reproductive Medicine and the Practice Committee of the Society for Assisted Reproductive Technology, Round spermatid nucleus injection (ROSNI), *Fertility and Sterility*, 2008, vol. 90 (sup. 3), p. S199-201.
69. Y. K. Kurotaki & al., Impaired active DNA demethylation in zygotes generated by round spermatid injection *Human Reproduction*, 2015, vol. 30/5, p. 1178–1187.
70. Atsushi Tanaka, & al., Ninety babies born after round spermatid injection into oocytes : survey of their development from fertilization to 2 years of age, *Fertility and Sterility*, 2018, vol. 110/3, p. 443-451.
71. Théo Meister & al., Association of Assisted Reproductive Technologies With Arterial Hypertension During Adolescence, *Journal of the American College of Cardiol*ogy 2018, vol. 72/1, p. 1267-1274.

independently of any parental project. This would also make it possible to bring an end to the controversies concerning the moral status of the EMSUs, whose creation could prove superfluous, given that it is partially due to the ban on creating embryos for research that the production of these models developed in the first place. Would it not be more scientifically judicious to create embryos on which studies could be performed? Consider the research of Ali Brivanlou on the chimeric models discussed above, with the aim of better understanding how the human embryo is organized during the appearance of the primitive streak, research conducted on chimeric embryos precisely in order to avoid the controversies related to experimental studies on human embryos[72]. Would it not be more appropriate to create embryos directly for such research, because they concern human beings and only them? Evidently, the issue deserves to be raised and debated.

72. Ian Martyn & al., *art. cit.,* p. 1 : « Owing to the ethical limitations of working with early human embryos, the only way to search for the human organizer is via human embryonic stem cells (hESCs) ».