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NEW DEBATE

Human embryo: a biological definition

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This paper defines a human embryo from a biological standpoint that takes into account emerging technologies in reproductive science. The paper does not consider legal, moral, religious or social views. As the definition of a human embryo must reflect the multifactorial processes of development, an approach has been adopted which combines recognition of observed events with potential for further development. This acknowledges that fertilization and development are not static processes, and as such embryo status can only be defined by observation of specific markers. The following biological definition of 'human embryo' is proposed.

A human embryo is a discrete entity that has arisen from either:

(i) ***the first mitotic division when fertilization of a human oocyte by a human sperm is complete or***
(ii) ***any other process that initiates organized 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.

Key words: definition/human embryo/primitive streak/syngamy

Introduction

Definitions of a human embryo normally include those entities created by the fertilization of a human oocyte by a human sperm. However, there have been a number of recent technological developments that have made it possible to create entities called embryos by other means, such as somatic cell nuclear transfer (SCNT) and induced parthenogenesis. Due to these examples and developing technologies, it was considered appropriate to revisit the biological definition of a 'human embryo'.

The last decade has seen the development of reproductive technologies that have resulted in considerable debate as to whether the entities that can, or could theoretically be generated would fall within current definitions of an embryo. Definitions based on a potential for further development might capture entities that might not be covered by definitions that specify a critical early developmental time point (e.g. completion of fertilization). For example, since some of the technologies do not involve fertilization, it has been proposed that the entities produced may not be considered to be embryos under some legal definitions (Morgan and Ford, 2004). This has been argued even though in some cases there is the possibility that if placed into the correct uterine environment, a viable individual could theoretically be produced. To date, there is no credible evidence of any cloned human beings having been born. However, the fact that in several

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Table I. The developmental potential and genetic contribution of entities produced either by natural processes of fertilization or as a result of emerging technologies in reproductive science

Reproductive technique	Male gamete	Female gamete	Functional element									Genetic contribution	
			Fertilization	Syngamy	Cleavage	Morula	Blastocyst	Potential to implant	Gastrulation	Potential to develop into a foetus	Potential for live birth	Nucleus	Mitochondria
Processes that occur naturally in humans													
Fertilization—naturally occurring	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Chimaera—embryo fusion[a,b]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Embryo splitting—monozygotic twins	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
Experimental techniques that have been successfully conducted using human material (italics indicate theoretical assessments as the entity has not been demonstrated experimentally to proceed to the developmental stage indicated)													
(1) Cloning by embryo splitting[c,d]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Both gamete donors	Oocyte donor
(2) Somatic cell nuclear transfer (SCNT)—human somatic cell and human oocyte[e]	No	No (enucleated oocyte)	No	No	Yes	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	Somatic cell donor	Oocyte donor
(3) Heterologous nuclear transfer—human embryonic stem (hES) cell nucleus and human oocyte[f]	No	No (enucleated oocyte)	No	No	Yes	Yes	Yes	*Yes*	*Yes*	*Yes*	*Yes*	hESC	Oocyte donor
(4) Pronuclear transplantation—transfer of pronuclei from fertilized human oocyte to enucleated donor human oocyte[g]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	*Yes*	Donors of gametes used for fertilization	Oocyte donor
(5) Parthenogenesis—human oocyte activation[e]	No	Yes	No	No	Yes	Yes	Yes	*No*	*No*	*No*	*No*	Oocyte donor	Oocyte donor
(6) Chimaera—generated by aggregation of individual viable blastomeres obtained from non-viable embryos[h]	No	No	No	No	Yes	Yes	Yes	*Yes*	*Yes*	*Yes*	*Yes*	Multiple origin depending on origin of blastomeres	Multiple origin depending on origin of blastomeres
Experimental technique that has been successfully conducted using human and animal material													
(7) SCNT—human somatic cell and enucleated animal oocyte[i,j]	No	No	No	No	Yes	Yes	Yes	*?*	*?*	*?*	*?*	Human somatic cell donor	Animal oocyte donor
Experimental techniques that have been successfully conducted in animal models involving no human material													
(8) Fertilization—mouse sperm generated *in vitro* from differentiating mouse embryonic stem (mES) cells[k]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Derived from mES cells used to generate sperm	Mouse oocyte donor
(9) Gynogenesis—as for pronuclear transplantation but using two maternal pronuclei[l,m]	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Oocyte donor	Oocyte donor
(10) Androgenesis—as for pronuclear transplantation but using two paternal pronuclei[m,n]	Yes	No (enucleated oocyte)	No	Yes	Yes	Yes	Yes	Yes	*?*	*?*	No	Sperm donor	Oocyte donor
(11) SCNT—mouse somatic cell genetically altered to remove implantation potential and enucleated mouse oocyte[o]	No	No	No	No	Yes	Yes	Yes	No	No	No	No	Mouse somatic cell donor	Mouse oocyte donor

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(12) Chimaera—injection of mouse blastocyst with mES cells	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Host embryo or mES cells (but not in same cell)	mES cells and host blastocyst cells (but not in same cell)
Proposed and theoretically possible experimental techniques (italics indicate theoretical assessments as the technique has not been published as successfully conducted)													
(13) Fertilization—human gametes generated *in vitro* from differentiating hES cells[p,q,r]	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	Derived from hES cells used to generate sperm	Derived from hES cell used to generate oocyte
(14) Fertilization—human gametes produced *in vitro*[s]	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	Male and female human tissue donors	Female human tissue donor
(15) Fertilization—human oocytes produced by animals containing human ovarian tissue grafts fertilized with human sperm[t]	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	Male and female human tissue donors	Female human tissue donor
(16) SCNT—human somatic cell genetically altered to remove implantation potential and enucleated human oocyte (or oocyte generated *in vitro* from differentiating hES cells)	*No*	*No*	*No*	*No*	*Yes*	*Yes*	*Depends upon genetic alteration*	*No*	*No*	*No*	*No*	Human somatic cell donor	Human oocyte donor
(17) SCNT—human somatic cell genetically altered to remove implantation potential and enucleated animal oocyte	*No*	*No*	*No*	*No*	*Yes*	*Yes*	*Depends upon genetic alteration*	*No*	*No*	*No*	*No*	Human somatic cell donor	Animal oocyte donor
(18) Chimaera—injection of hES cells into animal blastocyst	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*?*	*?*	*?*	*?*	Host embryo or hES cells (but not in same cell)	hES cells and host blastocyst cells (but not in same cell)
(19) Chimaera—injection of animal ES cells into human blastocyst	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*Yes*	*?*	*?*	*?*	*?*	Host embryo or animal ES cells (but not in same cell)	Animal ES cells and host blastocyst cells (but not in same cell)

[a]Strain *et al.* (1998)
[b]Yu *et al.* (2002)
[c]Footnote 25 in Daar and Sheremeta (2002)
[d]Chan *et al.* (2000)
[e]Cibelli *et al.* (2001)
[f]Stojkovic *et al.* (2005)
[g]Zhang *et al.* (2003)
[h]Alikani and Willadsen (2002)
[i]Chen *et al.* (2003)
[j]Chang *et al.* (2004)
[k]Nayernia *et al*. (2006)
[l]Kono *et al*. (2004)
[m]Surani (1986)
[n]Barton *et al*. (1984)
[o]Meissner and Jaenisch (2005)
[p]Hubner *et al*. (2003)
[q]Toyooka *et al*. (2003)
[r]Geijsen *et al*. (2004)
[s]Bukovsky *et al*. (2005)
[t]Gook *et al*. (2003).

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mammalian species, such as mice, sheep and cows, SCNT has resulted in live births that developed into healthy adult animals would suggest that this could be achieved in humans.

When considering what defines an embryo in the light of recent technological advances, it is important that the definition does not become so wide as to encompass human cells or cellular structures that traditionally have not been previously considered to be embryos. For example, it has been argued (Bailey, 2001) that a human somatic cell, the nucleus of which theoretically could become incorporated into a live entity after much manipulation, as demonstrated by the success of SCNT, could be considered a potential embryo. Further, hydatidiform moles, which may have derived from an embryo, have traditionally not been considered to be embryos.

Table I summarizes the developmental potential and genetic constitution of entities produced as a result of emerging technologies in reproductive science as well as horizon technologies that are based on indications from the literature. For comparison, embryos arising from the naturally occurring reproductive process are also included. From the information presented it can be inferred that the emerging technologies could produce entities that:

(i) have no potential to implant or result in a live birth and/or
(ii) do not have a contribution of genetic information from a sperm and an oocyte and/or
(iii) may contain DNA from two different species.

It is instructive to examine these key differences between entities produced by the naturally occurring reproductive processes and the emerging technologies in order to determine whether the latter could be defined as a human embryo.

Discussion

Should the potential to produce a live birth form part of the biological definition of a human embryo?

Animal models have demonstrated that SCNT blastocysts have the potential to implant and develop to a live birth (Wilmut *et al.*, 1997). It is therefore reasonable to assume that human SCNT blastocysts also have the potential to develop into a viable individual if placed within the correct environment.

It has been demonstrated that transferring viable blastomeres from developmentally slow preimplantation embryos into an empty zona pellucida produces an aggregate preimplantation structure that can develop to the blastocyst stage, from which human embryonic stem cells can be derived (Alikani and Willadsen, 2002). Although it remains to be tested whether such aggregate blastocysts (reproductive technique 6) can implant and form a viable pregnancy, it is theoretically feasible.

In the mouse model, significant progress has been made in the generation of gametes from embryonic stem cells (Hubner *et al*., 2003; Toyooka *et al*., 2003; Geijsen *et al*., 2004; Lacham-Kaplan *et al*., 2005). The generation of fully functional male gametes from embryonic stem cells *ex vivo* has recently been demonstrated (Nayernia *et al*., 2006). Another approach has been to derive human oocytes *in vitro* from ovarian surface epithelial cells (Bukovsky *et al*., 2005). It is yet to be demonstrated whether human oocytes produced using these strategies (reproductive techniques 13 and 14) are able to be fertilized and develop to form viable pregnancies. The use of such gametes in fertilization may result in the development of blastocysts that theoretically have the potential for implantation and forming a viable pregnancy.

Another option is the generation of animals that produce human gametes. To date, it has been demonstrated that mice containing a human ovarian xenotransplant can produce human oocytes (reproductive technique 15; Gook *et al*., 2003). Human gametes could in theory also be made by chimeric animals produced by injecting human embryonic stem cells into animal blastocysts (reproductive technique 18). The use of gametes produced by grafted or chimaeric animals in fertilization theoretically could result in entities that are capable of implantation and forming a viable pregnancy.

There have been proposals to genetically alter the nucleus of the somatic cell before transfer into an enucleated donor oocyte in a manner that would remove the implantation potential of any resulting human embryo clones (reproductive techniques 16 and 17). This technique has recently been demonstrated in the mouse model (Meissner and Jaenisch, 2005). Briefly, the donor cells were genetically altered to disrupt the expression of a gene that is essential for the formation of a functional trophoblast. The resultant entities formed inner cell masses, from which embryonic stem cells could be derived, but were unable to implant into the uterus. It has been argued that this technique, otherwise known as altered nuclear transfer, circumvents the ethical objections to using SCNT for the generation of human embryonic stem cells (Melton *et al*., 2004; Hurlbut, 2005a, b; Pacholczyk and Hurlbut, 2005). To date, there are no reports that this technique has been successfully conducted in humans.

Gynogenetic and androgenetic preimplantation embryos have only a paternal or a maternal genetic contribution, respectively (reproductive techniques 9 and 10). Such uniparental preimplantation embryos can be created by pronuclear transplantation. Androgenetic preimplantation embryos can also occur without experimental manipulation and result in what is known as a partial or complete hydatidiform mole depending upon morphology and genetic origin. Pathologically indistinguishable hydatidiform moles can also be biparental. A mutation in a gene, belonging to a protein family involved in inflammatory responses and programmed cell death, which causes recurrent hydatidiform moles in humans has been identified (Murdoch *et al*., 2006). Although androgenetic and gynogenetic preimplantation embryos may be able to develop to the blastocyst stage and implant, they are not able to establish viable pregnancies.

Parthenogenic preimplantation embryos (reproductive technique 5) are also uniparental as they have only a maternal genetic contribution. Although they can implant, they have limited subsequent developmental potential. In mice, parthenogenic embryos with potential to develop into a viable individual can be produced, but only after a substantial amount of genetic manipulation (Kono *et al*., 2004). In mice (Mann *et al*., 1990; Allen *et al*., 1994) and macaques (Vrana *et al*., 2003), it has been demonstrated that parthenogenic preimplantation embryos

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can develop to the blastocyst stage and are amenable to the generation of embryonic stem cells. In humans, however, parthenotes are unlikely to develop beyond the first few divisions, as the centrioles contributed by the human sperm are required for the formation of a functional centrosome (Pickering *et al*., 1988).

Most of the emerging technologies summarized in Table I produce entities that have the potential to implant and form a viable pregnancy. Indeed, it is likely but not proven that should they be allowed to develop to term, live births would result. It is therefore reasonable to conclude that if these techniques are conducted using human material, they could produce a live human being.

Some of the emerging technologies discussed above produce entities with no potential to form a viable pregnancy. From a purely biological perspective, conducting these techniques using only human material would produce a human blastocyst but not a viable pregnancy or live birth. If the potential to produce a live birth is to be a key element of a definition of human embryo, then gynogenesis and androgenesis (reproductive techniques 9 and 10) would not be considered to be techniques that can produce a human being, even if only human material is used.

The above discussion suggests that the potential to form a new living being may indeed be a useful component of a definition of 'human embryo', as it allows a distinction between emerging technologies that may lead to live births from those that do not.

Should fertilization and/or syngamy form part of the biological definition of human embryo?

A number of the emerging technologies summarized in Table I do not involve the contribution of chromosomal DNA by both a sperm and an oocyte or the completion of syngamy (reproductive techniques 2, 5–7, 9–11 and 16–17). However, some of these techniques, if conducted using human materials, might have the potential to produce a live human birth. Given this, it would be expected that a human embryo would be created during the developmental processes initiated using these techniques. The inclusion of fertilization and syngamy as necessary elements in a definition of 'human embryo', would eliminate emerging technologies that have the potential (even if theoretical at present) to produce a new human being. Therefore, an absolute requirement for fertilization and/or syngamy may not be appropriate for the biological definition of a human embryo.

Should the biological definition of human embryo exclude techniques combining DNA from more than one species?

Some emerging technologies could theoretically result in an entity with a nuclear genome that is human while the mitochondrial genome could be derived from another species (reproductive technique 7). It is not known whether mitochondrial heteroplasmy would cause developmental problems (Brenner *et al*., 2004). This is an unresolved aspect of SCNT, as it is possible that cloned embryos will contain mitochondria from different sources, i.e. associated with the transplanted donor nucleus and also from the recipient host-enucleated oocyte.

Another possibility is an entity that contains cells from different species. Injection of genetically altered mouse embryonic stem cell lines into mouse blastocysts is used to generate transgenic and knockout mice (reproductive technique 12). Since the embryonic stem cell lines were derived from a different individual to the host blastocyst, a chimaera is produced. It is not clear whether this technique could be applied to the generation of interspecific chimaeras. Transplantation of whole rat inner cell masses or individual rat inner cell mass cells into mouse blastocysts did not produce any viable live births (Gardner and Johnson, 1975). Therefore, the developmental potential of chimaeras created by injecting human embryonic stem cells into a blastocyst from a different species (reproductive technique 18) or by injecting non-human embryonic stem cells into a human blastocyst (reproductive technique 19; DeWitt, 2002) is unknown.

Some of the techniques included in Table I have the potential to produce an entity with DNA from more than one species. Any technique that could result in a live birth would likely involve the formation of an embryo at some point in the early development process. Therefore, the biological definition of a human embryo should not specifically exclude an entity created with DNA from two species.

Should the biological definition of human embryo include a developmental time point?

It has been previously argued that the potential for continued development should be a key consideration for any definition of 'embryo' (Latham and Sapienza, 2004). The discussion presented in this paper fully supports this view. However, it is questionable whether it is possible to define 'human embryo' without making some reference to a developmental point in time.

Another approach to the development of a biological definition of 'human embryo' may be one that does include a reference to a specific developmental time point, but in the context of the potential for continued development. The term 'human embryo' is not applicable before the completion of fertilization of a human oocyte by a human sperm (i.e. syngamy), because this is when the new genome of the new individual is created. Prior to syngamy the maternally and paternally inherited genomes exist as two separate genomes.

A definition of 'human embryo' based on syngamy excludes reproductive technologies that do not involve the fertilization of a human oocyte by a human sperm. Although some of these technologies might result in live births if applied to the human, it is clear from animal studies that others would not. From a biological perspective, setting the definitive time point at syngamy would include entities that have no potential to form a live human individual. It may be more appropriate to assess the potential of such entities to develop to, or beyond the appearance of, the primitive streak.

The above discussion suggests that a definition of 'human embryo' may need to be separated into two components: one for early developmental processes resulting from the fertilization of a human oocyte by a human sperm and the second for those resulting by other means.

A final consideration is whether the definition should refer to syngamy, which cannot be visually confirmed until the initiation of the first mitotic division. Given that the aim of this paper is to develop a biological definition of 'human

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Table II. Emerging technologies and their status under the biological definition of human embryo

Reproductive technique	Covered by definition?
Processes that occur naturally in humans	
Fertilization—naturally occurring	Yes
Chimaera—embryo fusion	Yes
Embryo splitting—monozygotic twins	Yes
Emerging technologies	
(1) Cloning by embryo splitting	Yes
(2) SCNT—human somatic cell and human oocyte	Yes
(3) Heterologous nuclear transfer—hES cell nucleus and human oocyte	Yes
(4) Pronuclear transplantation—transfer of pronuclei from fertilized human oocyte to enucleated donor human oocyte	Yes
(5) Parthenogenesis—human oocyte activation	Insufficient information available
(6) Chimaera—generated by aggregation of individual viable blastomeres obtained from non-viable embryos	Yes
(7) SCNT—human somatic cell and enucleated animal oocyte	Yes
(8) Fertilization—mouse sperm generated *in vitro* from differentiating mES cells	Yes
(9) Gynogenesis — as for pronuclear transplantation but using two maternal pronuclei	Insufficient information available
(10) Androgenesis — as for pronuclear transplantation but using two paternal pronuclei	Insufficient information available
(11) SCNT—mouse somatic cell genetically altered to remove implantation potential and enucleated mouse oocyte	No (no human material involved)
(12) Chimaera—injection of mouse blastocyst with mES cells	No (no human material involved)
(13) Fertilization—human gametes generated *in vitro* from differentiating hES cells	Yes
(14) Fertilization—human gametes produced *in vitro*	Yes
(15) Fertilization—human oocytes produced by animals containing human ovarian tissue grafts fertilized with human sperm	Yes
(16) SCNT—human somatic cell genetically altered to remove implantation potential and enucleated human oocyte (or oocyte generated *in vitro* from differentiating hES cells)	No
(17) SCNT—human somatic cell genetically altered to remove implantation potential and enucleated animal oocyte	No
(18) Chimaera—injection of hES cells into animal blastocyst	Insufficient information available
(19) Chimaera—injection of animal ES cells into human blastocyst	Insufficient information available

embryo' it may be preferable to include a measurable event, such as the first mitotic division.

The biological definition of human embryo

After consideration of the issues raised in the preceding discussion, the following biological definition of 'human embryo' is proposed.

A human embryo is a discrete entity that has arisen from either:

(i) *the first mitotic division when fertilization of a human oocyte by a human sperm is complete or*

(ii) *any other process that initiates organized 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.

This definition attempts to combine the aspects of observed stages of development, developmental potential and origin of the DNA contributing to the new individual. It is recognized that this definition creates the possibility of an anomaly whereby an entity which arose from completion of fertilization of a human oocyte by a human sperm and, for whatever reason, lacked the potential for future development would be considered as an embryo, whereas an identical entity that was artificially created would not have the status of an embryo. However, completion of fertilization of a human oocyte by a human sperm is sufficient to define an entity as a human embryo regardless of any potential, or lack thereof, for future development.

Having arrived at the biological definition of a human embryo, it is instructive to apply it to the emerging technologies previously discussed (Table II).

Conclusion

Naturally occurring early human developmental processes as well as emerging technologies in reproductive sciences have been considered in this discussion paper. The deliberations focused on the biology of these processes and technologies. On the basis of these facts, a biological definition of 'human embryo' was arrived at. The definition specifies that the term 'human embryo' cannot be applied prior to the completion of syngamy, or after 8 weeks of development. The biological definition of 'human embryo' presented in this discussion paper also acknowledges that emerging reproductive technologies may one day provide alternatives to the presently available reproductive techniques (e.g. *in vitro* fertilization, intracytoplasmic sperm injection). From a purely biological perspective, it is clear that the application of such technologies would produce new individuals that at some point in the developmental process would have been a human embryo.

The definition does not specify how much human genetic content an entity must possess before it can be considered to be a human embryo. It is felt that this issue would be more effectively addressed in the future as at present there is limited biological information. Until such time, the 'humanness' of a genome should be considered on a case-by-case basis.

It was beyond the scope of this discussion paper to consider legal, ethical and moral ramifications of these emerging technologies. However, it is hoped that when relevant experts undertake such considerations they may use this paper as a source of information.

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