Embryo-like entities: A commentary

Strategic delivery:	☒ Safe, ethical, effective treatment	☐ Consistent outcomes and support	☐ Improving standards through intelligence
Details:			
Meeting	Scientific and Clinical Advances Advisory Committee (SCAAC)		
Agenda item	7		
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Authors	Rasheda Begum, Scientific Policy Officer Anna Quinn, Scientific Policy Manager Andy Greenfield, Authority member Robin Lovell-Badge, SCAAC external advisor		
Output:			
For information or decision?	For discussion		
Recommendation	Members are asked to: • Discuss the scientific and potential clinical applications or ELEs described and evaluate their similarity to standard embryos • Highlight any other methods that can be used to derive ELEs which are not mentioned in this paper		
Resource implications	None		
Implementation date	N/A		
Communication(s)	None		
Organisational risk	☒ Low	☐ Medium	☐ High

1. Introduction

1.1. As the UK regulator of fertility treatment and human embryo research, the HFEA carefully monitors developments in the field of reproductive science. The HFEA's Scientific and Clinical Advances Advisory Committee (SCAAC) provides insight into the scientific and clinical implications of developments in research, to support ongoing monitoring by the Executive.

1.2. In recent years there has been progress in research in the UK and internationally involving various novel structures ('embryo-like entities'). These embryo-like entities (ELEs) share some properties with standard embryos created by fertilisation of an egg derived from an ovary either through non-assisted conception or through conventional *in vitro* fertilisation (IVF) or intracytoplasmic sperm injection (ICSI). ELE is a broad term which encompass a range of structures, from embryonic stem cells, which share their developmental potential with some cells of the embryo, to self- organising biological structures, which share their physical structure and pattern with embryos and are created by co-culture of different types of stem cell in a 3-dimensional scaffold.

1.3. This paper outlines a spectrum of different ELE, methods for deriving ELE, and how ELE relate to the standard embryo as described in the HFE Act 2008 (as amended). The Act refers to the "meaning of an embryo" in the following terms:

> *"(a) embryo means a live human embryo and does not include a human admixed embryo (as defined by section 4A(6)), 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."*

Drafted some years ago, the Act was framed to cover embryos created either through regular unassisted conception, or created *in vitro* via assisted conception methods including IVF/ICSI. Some of the methods of creating ELE may involve generating eggs and sperm *in vitro*, while others consist of deriving ELE directly from various stem cell types. This is a fast-moving area of research and this paper is not intended as a comprehensive review of all the relevant scientific literature. Instead we consider to what extent ELE differ in their derivation, behaviours, and developmental potential to standard embryos.

1.4. This paper does not consider whether the different types of ELE fall under the regulatory scope of the HFE Act 2008 (as amended). Nor does it consider the ethical, legal or regulatory issues involved. Rather, its purpose is to provide a basis for a discussion of the scientific and clinical merits of different ELEs.

1.5. In writing this paper the Authority is interested to consider the potential future regulatory need around any research activity or storage which involves structures which share common features with embryos. SCAAC's discussions will therefore inform a broader discussion by the Authority about advances in this area of research and the extent to which, under the HFE Act 2008 (as amended), the HFEA can have, or should seek to establish, regulatory oversight of UK research creating, storing or using different categories of embryo-like entities.

2. *In vitro* derived gametes

2.1. *In vitro* derived gametes are germ cells (egg and sperm) which have been derived from embryonic stem cells, induced pluripotent stem cells, or even already differentiated cells. Under specific

culture conditions, stem cells or somatic cells can be programmed to differentiate into certain cell types (e.g. egg and sperm) which are required for reproduction. There is significant interest in use of *in vitro* derived gametes for treatment of infertility. *In vitro* derived gametes were highlighted as a high priority issue for SCAAC in 2016, and a literature review was therefore considered at the October 2016 meeting. It is important to note that whilst the HFE Act 1990 (as amended) permits the creation and use of *in vitro* derived gametes in research, it does not permit the use of *in vitro* derived gametes in treatment.

2.2. Research into the possibilities for *in vitro* derived gametes have been largely limited to mice. This has been addressed by SCAAC, who questioned whether the mouse model is an accurate model for human development. A recent study by Gomes Fernandes et al., 2018 examined expression of certain biomarkers in a 4.5-week-old human embryo and found that genes known to be expressed in mouse primordial germ cells (PGCs) were expressed differently in human PGCs.

2.3. Fertilisation has been achieved using *in vitro* derived gametes in mice, as seen in a study by Morohaku et al., 2016 where fertile eggs were generated from primordial germ cells. Fertilisation of these eggs with epididymal sperm led to the birth of 7 pups. A study by Zhou et al., 2016 demonstrated generation of *in vitro* derived spermatids which, when injected into eggs, produced viable and fertile offspring. Complete generation of i*n vitro* derived sperm cells has not been achieved.

2.4. It may be argued that the ELEs generated by combining *in vitro* derived gametes would have a similar status to embryos since the process of fertilisation would be involved.

2.5. There is no evidence from either animal or human studies to suggest that completely *in vitro* derived eggs and sperm used in combination have the capability to undergo the process of fertilisation. The developmental potential of an ELE created in this way has therefore never been demonstrated.

3. Organoids

3.1. The formation of organoids involves culturing stem cells which develop into structures that resemble tissues and organs. This has been attempted widely for non-reproductive organs; research has shown potential for use in regenerative treatment, for example, human optic cup organoids have been engrafted into injured eyes (Shirai et al., 2016) and liver organoids have been observed to recover mice from acute liver failure (Nie et al., 2018). Organoids have also been established as model systems for studying effect of diseases, as one study used human pluripotent stem cells to create organoids resembling structures in the human brain and infected them with Zika to find receptors susceptible to Zika virus with the aim to provide insight into drug therapies (Watanabe et al., 2017). Within the context of fertility, some studies have shown the possibility of developing *in vitro* derived gonads.

In vitro derived gonads

3.2. *In vitro* derived gonads are another avenue for potentially creating ELEs. There has been progress in development of *in vitro* derived ovaries in mouse studies. A study by Chiti et al., 2018 cultured isolated immature mouse follicles and these were grown in a scaffold. After transplantation these structures were able to survive and grow. Another study demonstrated development of a biosynthetic ovary which was implanted in to sterilised mice; the mice went on to have live births by spontaneous conception (Laronda et al., 2017).

3.3. Research into developing gonadal organoids has led to noteworthy results in mouse studies, as testicular tissue has been formed that can also produce sperm (Sato et al., 2011).

3.4. One human study took testicular cells from both adult males and pubertal males. The cells were able to reorganise themselves into testicular organoids which could produce testosterone (Baert et al., 2018).

3.5. There needs to be further research into differentiation of stem cells into testicular cells as indicated by Alves-Lopez and Stukenborg (2018), which would provide a further challenge in being able to derive viable embryos.

3.6. By generating gonads *in vitro*, researchers open the possibility of creating ELEs *in vivo* if the structures are placed in the body. Animal studies have already demonstrated that embryo-like entities created in this way have the potential to develop into live offspring. It might therefore be proposed that these embryo-like entities share the same status as embryos.

4. Self-organising structures

4.1. Culturing stem cells and applying signals can cause these cells to self-organise into ELEs, as structures that resemble embryos. To date, several different structures have been identified in research:

Embryoids

Embryoids may be generated from embryoid bodies which consists of 3D aggregates of embryonic stem cells. One type of embryoid is a blastoid, which resembles a blastocyst with the features that are indicative of a blastula, including cavity formation as shown in a study by Rivron et al., 2018, where a blastoid structure resembling a 3.5-day embryo was generated from stem cells. In a study by Okae et al., 2018, trophoblast cell lines were derived from human blastocysts. These cell lines could possibly be used to make human blastoids in conjunction with stem cells.

Gastruloids

Another type of structure identified is the gastruloid that resembles the gastrula, which is the stage of embryonic development where three distinct layers form that give rise to various cells and tissues (Warmflash et al., 2014).

ETS-embryos

In a study by Harrison et al., 2017 stem cells were used to create ELEs, embryo-like structures. The authors found that they were able to recapitulate the morphogenesis of embryos, i.e. mimic how their shape develops. These structures were termed ETS-embryos, as they were made using mouse embryonic stem cells (ESCs) and extraembryonic trophoblast cells (TSCs).

4.2. ELEs may also be created by using 3D bioprinting. This method has a more mechanical approach compared to other methods discussed in this paper, which use chemical signals. In a study by Warmflash et al. 2014, stem cells were grown within a disc shaped mould, allowing control of precise spatial patterning.

4.3. The structures above are created with procedures that do not use gametes in any way. While they may share the appearance of early embryos, their origins are divergent from that of embryos and their development potential has not been demonstrated.

Hybrid embryos

4.4. In a study by Martyn et al. (2018), ELEs were formed from human embryonic stem cells that self-organised into early embryonic germ layers and, after induction with Wnt and Activin, transcription analysis showed that the structures expressed genes that were found in organisers (cells that induce and pattern adjacent embryonic cells). The structures were grafted into chicken embryos and this led to a secondary axis being formed and a secondary nervous system.

4.5. A hybrid embryo as described above does not arise from fertilisation and combines two species, therefore it would be removed from the status of a standard embryo. The study by Martyn et al. took place in the USA. In the UK creation of a hybrid embryo requires an HFEA licence and hybrid embryos created using human and animal material cannot be implanted into a woman or allowed to develop for more than 14 days. The HFEA has not yet considered whether the kind of hybrid embryos created by Martyn et al. would meet the definition in the HFE Act.

5. Embryonic stem cells

5.1. Embryonic stem cells (ESCs) are pluripotent stem cells obtained from the inner cell mass (ICM) of an embryo. ESCs are ELEs which have been used extensively to study embryonic development.

5.2. The existence of ESCs with wider potency has been established. In a study by Yang et al., 2017, a specific chemical cocktail was used to derived stem cells with extended pluripotency. These stem cells were able to generate embryonic and extraembryonic tissues.

5.3. It has also been observed that within pluripotent mouse embryonic stem cell cultures, there are a population of cells which resemble 2-cell stage embryos. At the 2-cell stage, cells are still totipotent which gives an indication that 2-cell-like cells could have similar development potential. Studies have shown 2-cell-like cells express embryonic and extraembryonic determinants (Morgani et al., 2013, Martin Gonzales et al., 2016).

5.4. It remains unknown whether these stem cells can be maintained in isolation or could give rise to a new-born mouse if placed in a foster mother (Baker and Pera, 2018). In any case, fertilisation would not take place therefore any embryo-like structures derived would differ from standard embryos.

6. Conclusions

6.1. Research using ELEs is clearly complex and encompasses a broad range of structures which are derived using a variety of different methods. To determine how closely ELEs relate to standard embryos, it may be helpful to consider the similarities and differences between their methods of creation. For example, *in vitro* derived gametes and *in vitro* derived gonads require the process of fertilisation to create ELEs. However, creation of ELEs using stem cells which self-organise, either by 3D-bioprinting or chemical signals, does not require a fertilisation step at all. It is also important to consider the developmental potential of ELEs, for example animal studies have shown that ELEs created using in vitro derived gonads have the potential to develop into living beings, whereas, the developmental potential of self-organising structures has not been demonstrated.

6.2. In order to consider the regulatory and ethical status of ELEs it could be beneficial to establish a schema, possibly expressed as a spectrum which outlines the extent to which these structures are

analogous to embryos, whether in their methods of derivation, structure, or developmental potential.

6.3. Members are asked to:

- Discuss the scientific and potential clinical applications of ELEs described and evaluate their similarity to standard embryos
- Highlight any other methods that can be used to derive embryo-like entities not mentioned in this paper

7. References

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