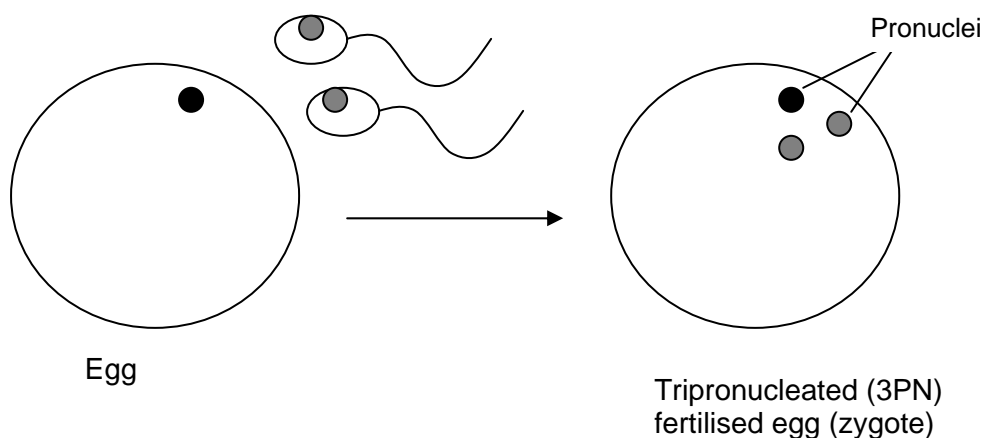


**Human Fertilisation and Embryology Authority  
The Scientific and Clinical Advances Group**

<b>Committee:</b>	Scientific and Clinical Advances Group
<b>Meeting Date:</b>	21 <sup>st</sup> May 2008
<b>Agenda Item:</b>	7
<b>Paper Number:</b>	SCAG(05/08)03
<b>Paper Title:</b>	Use of trippronucleated (3PN) embryos
<b>Author:</b>	Helen Richens
<b>For Information or Decision?</b>	Decision
<b>Resource Implications:</b>	Accounted for in the business plan
<b>Recommendation to the Committee:</b>	Members are asked for their views on: <ol style="list-style-type: none"> <li>1. whether removing a pronucleus from 3PN embryos is feasible for reproductive purposes</li> <li>2. whether clinics are likely to want to use this technique and form a view on whether it should be a licensed activity if used for reproduction</li> <li>3. applications of 3PN embryos for research purposes</li> </ol>
<b>Annex:</b>	Annex A: Escribá et al (2006) Heteroparental blastocyst production from microsurgically corrected trippronucleated human embryos. <i>Fertility and Sterility</i> 86(6): 1601-1607.

### Lay summary

Sometimes during IVF two sperm can fertilise an egg at the same time. This leads to the fertilised egg (zygote) containing a nucleus from the egg and two nuclei from the two sperm. The nuclei at this stage are called pronuclei and therefore the zygote is said to be 'trippronucleated' (3PN).



The pronuclei contain the egg and sperm's DNA. A normally fertilised zygote has one set of genetic material from the pronuclei of the egg and one set of genetic material from the pronuclei of the sperm. A 3PN zygote has an extra set of genetic material. 3PN zygotes occur in about 4-7% of IVF cycles and are automatically discarded from IVF treatment.

Embryologists are becoming increasingly skilled at identifying and removing the extra pronucleus. This means potentially 3PN embryos could be corrected and used in IVF treatment cycles, instead of being discarded.

Some researchers have also been working on using 3PN zygotes in cloning experiments. These could potentially be used to derive patient-specific embryonic stem cell lines, instead of using viable embryos.

## 1. Background

1.1. This issue was identified during the 2007-08 horizon scanning process and identified as a high priority issue.

## 2. Use of 3PN embryos for treatment

2.1. 3PN embryos can arise from fertilisation of oocytes by diploid (nonreduced) spermatozoa, or from dispermic fertilisation.

2.2. Altering the ploidy and parental constitution of zygotes has been carried out efficiently in several mammalian species. Until recently groups had been able to remove a pronucleus to create bipronucleated diploid human embryos, but the *in vitro* developmental ability of these embryos was restricted (Gordon *et al.* 1989, Malter *et al.* 1989). The heteroparental inheritance of the embryo was also uncertain.

2.3. Recently however a Spanish group Escribá *et al.* (2006) developed a technique that improves identification and removal of the extra paternal pronuclei. The group microsurgically removed the pronucleus located furthest from the second polar body in 3PN human zygotes using cytoskeletal relaxing agents (see paper in Annex A). The resulting embryos were diploid and developed to blastocyst stage, with the majority being heteroparental. The group think that this technique could be useful for reproductive purposes or to provide embryos for embryonic stem cell research.

2.4. There has been one case report in Singapore (Kattera & Chen 2003) of corrected 3PN human embryos being used for reproductive purposes, resulting in a live birth. The child appeared developmentally normal.

2.5. 3PN embryos are currently discarded by clinics from use in IVF treatment cycles. Between 4% and 7% of fertilised eggs have been reported as being 3PN zygotes in IVF cycles. The technique could impact patients seeking fertility treatment by increasing the number of embryos available for transfer or by allowing more embryos to be frozen for use in additional treatment cycles.

### **3. Use of 3PN embryos for nuclear transfer research**

- 3.1. One US group Egli *et al.* (2007) has also recently used 3PN mice zygotes as recipients in nuclear transfer. The group generated zygotes fertilised with two spermatozoa. They used inhibitors to temporarily block the cell during mitosis, then removed all chromosomes in the zygote and replaced them with chromosomes from a donor ES cell. When the inhibitors were removed, development resumed and a blastocyst formed. These blastocysts could potentially be used to derive new ES cell lines.
- 3.2. 3PN zygotes could therefore potentially be an alternative source of embryos for human embryonic stem cell research. Information on this has been included in the literature review on alternative ways to derive embryonic stem cells, which is being passed on to research licence committees.

### **4. Timescale of technique**

- 4.1. The technique has already been used for reproductive purposes abroad and improvements in the technique have recently been shown in human embryos. Therefore a clinic may apply to the HFEA to use the technique for reproductive applications in the relatively near future.
- 4.2. The potential for 3PN embryos to be used in stem cell derivation has been explored in the recent paper by Egli *et al.* (2007). However this experiment was conducted in mice and has not been transferred to humans yet.
- 4.3. 3PN embryos have been used for other research purposes. For example the HFEA has already licensed one research project at St Mary's Manchester involving poly-pronucleated embryos: R0026 In vitro development and implantation of normal human preimplantation embryos and comparison with uni- or poly-pronucleated pre-embryos.

### **5. Implications of the technique**

- 5.1. The issue has potential public interest because it could increase the number of embryos available for transfer in IVF treatment cycles. Micromanipulation of embryos raises safety concerns which would have to be addressed. Because the parental origin of the removed pronucleus could severely affect the reproductive outcome, embryo biopsy would have to be carried out before embryo transfer to identify the parental inheritance of the embryo.
- 5.2. Some ethical concerns may be raised about techniques that alter the parental constitution of embryos.
- 5.3. The technique could be used to generate ES cell lines from embryos that would otherwise have been discarded. This may alleviate some ethical concerns about using embryos for stem cell research.

### **6. Relevant legislation**

- 6.1. The use of 3PN embryos is not prohibited by current legislation or HFEA policy.

- 6.2. Paragraph 3(4) of Schedule 2 to the Human Fertilisation and Embryology (HFE) Act 1990 specifically prohibits altering the genetic structure of an embryo. The Licence Committee initially rejected a licence application by Newcastle Fertility Centre at Life (R0153 Mitochondrial DNA disorders: is there a way to prevent transmission?) on the grounds that pronuclear transfer would alter the genetic structure of the embryo. However the appeal committee did not consider this to apply to pronuclear transfer. Removing a pronucleus may alter the genetic constitution or composition of a 3PN embryo, but it does not alter the genetic structure.
- 6.3. Under the Human Fertilisation and Embryology Bill a permitted egg or embryo for treatment cannot have its nuclear or mitochondrial DNA altered.
- 6.4. Currently Standard S.7.8.6 in the Code of Practice requires micromanipulation of embryos to be carried out by a person authorised to carry out the procedure, for a purpose authorised by the Centre's licence. This relates to paragraph 1(2) of Schedule 2 of the HFE Act 1990.
- 6.5. Clinics wanting to micromanipulate 3PN zygotes to create diploid embryos for use in IVF treatment would need to inform the HFEA as it is a new activity. Groups wanting to use 3PN embryos for stem cell research will require a research licence from the HFEA.

## 7. Conclusions

7.1. Members are asked for their views on:

- whether removing a pronucleus from 3PN embryos is feasible for reproductive purposes
- whether clinics are likely to want to use this technique and form a view on whether it should be a licensed activity if used for reproduction
- applications of 3PN embryos for research purposes

## 8. References

- Egli D et al (2007) Developmental reprogramming after chromosome transfer into mitotic mouse zygotes. *Nature* 447: 679-686.
- Escribá M et al. (2006) Heteroparental blastocyst production from microsurgically corrected tripronucleated human embryos. *Fertility and Sterility* 86: 1601-1607.
- Gordon JW et al (1989) Successful microsurgical removal of a pronucleus from tripronuclear human zygotes. *Fertility and Sterility* 52: 367-72.
- Ivakhnenko V et al (2000) A microsurgical technique for enucleation of multipronuclear human zygotes. *Human Reproduction* 15(4): 911-916.
- Kattera S & Chen C (2003) Normal birth after microsurgical enucleation of tripronuclear human zygotes: Case report. *Human Reproduction* 18(6): 1319-1322.
- Malter HE & Cohen J (1989) Embryonic development after microsurgical repair of polyspermic human zygotes. *Fertility and Sterility* 52: 373-380.