

Human Fertilisation and Embryology Authority Scientific and Clinical Advances Group

Committee:	Scientific and Clinical Advances Group
Meeting Date:	14 th February 2006
Agenda Item:	7
Paper Number:	SCAG (02/06)0 and Annex A
Paper Title:	Inadvertent transgenesis caused by ICSI
Author:	Katy Berry (Policy manager)
For Information or Decision?	Information
Resource Implications:	None unless there are particular concerns raised following discussion of the paper that require follow-up.
Recommendation to the Committee:	Members are asked to: <ul style="list-style-type: none"> • discuss the data presented in the paper and; • comment on the implications of this work with respect to safety of ICSI and IVF

1. Background

1.1 When sperm is incubated with DNA the sperm is able to take-up DNA and upon fertilisation the exogenous DNA is transmitted into the oocyte simultaneously with the sperm genetic material. This is known as sperm mediated gene transfer (SMTG) and results in a transgene being present in the embryo. SMGT is used routinely as a technique to produce transgenic animals in research. SMGT can occur with treated sperm following artificial insemination, IVF and ICSI.

1.2 A paper was recently published looking at the transmission of bacterial DNA into the embryo following intracytoplasmic sperm injection (ICSI) in mice. The paper (Annex A) raises concerns about 'inadvertent transgenesis' or SMTG specifically as a result of ICSI.

2. Summary of findings

2.1 The group compared transgenesis of frozen-thawed sperm exposed to bacteria and exogenous DNA, frozen-thawed sperm exposed to exogenous DNA only from the washing medium and fresh sperm (washed in a Percoll gradient)

exposed to bacteria and exogenous DNA. In all comparisons fertilisation was achieved by ICSI.

2.2 Following ICSI, mouse embryos were analysed for expression of the transgene which in this case was a construct that caused the expression of a Green Fluorescent Protein (GFP) that can be visualised in the cells. The engineered construct has a strong constitutive promoter used widely in research to express large amounts of protein in mammalian cells. This promoter is unlikely to be present in any naturally occurring bacterial contamination that would be found in sperm samples and so any expression in the embryos is likely to be an over-representation of what might occur naturally.

2.3 By analysis of the embryos, the group demonstrated that the protein encoded by the exogenous DNA was expressed in 45 out of 237 (19%) embryos that reached the 2-cell stage that had been injected with frozen-thawed sperm exposed to bacteria containing the exogenous DNA. In embryos injected with sperm exposed to the washing medium of bacteria containing the exogenous DNA, 17 out of the 84 (20%) expressed the protein encoded by the exogenous DNA. For embryos injected with fresh sperm exposed to the exogenous DNA 10 out of 82 (12%) expressed the protein encoded by the exogenous DNA. The difference between the numbers of embryos that expressed the GFP encoded by the exogenous DNA was not significant. The expression of the GFP in the embryos demonstrates that the exogenous DNA is present in the embryo and is being expressed (made into protein).

2.4 The group then went on to see if the presence of the exogenous DNA is maintained in the older embryo. Embryos that were injected with either fresh or frozen-thawed sperm exposed to the bacteria containing the exogenous DNA were replaced into mouse and allowed to develop to day 14 (this is equivalent to embryonic day 50 in humans). The embryos were then tested for the presence of the exogenous DNA – the transgene – using polymerase chain reaction (PCR). From the frozen-thawed sperm embryos, 9 out of 146 (6%) contained the exogenous DNA that encoded the GFP and 2 out of 146 (1%) contained the bacterial DNA. From the fresh sperm ICSI embryos none contained the exogenous DNA that encoded GFP and none contained bacterial DNA.

2.5 The group also noted that the presence of bacteria in the sperm sample that is used for ICSI compromised the development of the embryos. In embryos injected with frozen- thawed sperm contaminated with the bacteria the percentage of embryos that reached the two-cell stage was significantly lower than those embryos that were injected with sperm not exposed to the bacteria or those sperm that had been washed through a Percoll gradient.

2.6 Although mentioned in the introduction, the possibility of SMGT following standard IVF techniques was not analysed in this study. This would have been a

useful comparison to make as it is theoretically possible that transgenesis could occur in assisted reproduction techniques other than ICSI.

2.7 The group conclude that *'full bacteriological semen examinations and effective antibiotic semen processing, should be taken especially in human infertility clinics, in order to exclude any possibility of accidental transgenesis as the result of ICSI.'*

3. Conclusions

The data presented here is worth noting but should not cause undue concern because the data presented is likely to be an over-representation of anything that would occur in normal situations. This is because they used very high levels of bacteria that had an engineered constitutively active, strong, mammalian promoter that would not be found in naturally occurring bacteria.

A further consideration that was not covered in the study is that transgenesis could occur not only from ICSI but also from IVF alone. No data were presented about this in the study.

Finally, the group did not present any evidence of negative effects caused by transgenesis. If transgenesis were occurring it is likely to have been happening since the introduction of ART techniques and the group might have suggested a possible consequence of this.

3.1 Members are asked to:

- discuss the data presented in the study (Annex A) and the comments in this paper;
- comment on the implications of this work with respect to safety of ICSI and possibly IVF