

First use of DNA fingerprinting to identify viable embryos

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Fertility researchers have used DNA fingerprinting for the first time to identify which embryos have implanted after in vitro fertilisation (IVF) and developed successfully to result in the births of healthy babies. The technique, combined with sampling cells from blastocysts (the very early embryo) before implantation in the womb, opens the way to pin-pointing a handful of genes that could be used to identify those blastocysts most likely to result in a successful pregnancy.

The authors of the research, published online in Europe's leading reproductive medicine journal, *Human Reproduction*, today (Wednesday 14 May), believe that their findings will revolutionise IVF by improving pregnancy rates and eliminating multiple pregnancies.

When couples attend fertility clinics for IVF, eggs from the woman are fertilised with sperm from the man and then the fertilised eggs are allowed to develop in the laboratory until they reach the blastocyst stage after about five days [2]. Before the blastocysts are implanted into the woman's womb, a decision has to be made about how many should be implanted and which ones look most likely to develop successfully. Currently there is no reliable way of differentiating between viable and non-viable blastocysts, and clinic staff tend to decide on the basis of some fairly crude tests, which include looking at the form (morphology) of the blastocyst. The result is that couples often opt to have more than one blastocyst implanted in order to increase their chances of a successful pregnancy; but this runs the risk of multiple pregnancies with all the associated dangers to both the mothers and babies.

When multiple embryos are transferred, it then becomes impossible to work out which are the ones that developed into a successful pregnancy, making it difficult to develop criteria for identifying viable blastocysts.

One of the authors of the paper, Dr David Cram, senior research scientist at the Monash Immunology and Stem Cell Laboratories, Monash University, Australia, said: “DNA fingerprinting is the ultimate form of biological identification, but until now it has not been used to identify the embryonic origin of resultant babies born following embryo transfer; nor has it been used for gene expression studies. We have developed a novel strategy of utilising a combination of blastocyst biopsy, DNA fingerprinting and microarray analysis to identify viable blastocysts among the cohorts transferred to patients. Our ultimate aim is to find out which genes are expressed by viable blastocysts.”

The researchers from Monash University and Dr Kostas Pantos and Ms Georgia Kokkali from the Centre for Human Reproduction, Genesis Athens Hospital, Athens, Greece, recruited 48 women undergoing in vitro fertilisation treatment, and after eggs were fertilised and developed in culture for five days, removed between eight and 20 cells from the trophoctoderm cell layer of the resulting blastocysts. These samples were amplified and their gene expression analysed using microarrays (a method of using genetic probes on a microchip to target sequences of messenger RNA [3]). One or more blastocysts were transferred to all 48 women and 25 became pregnant, with 37 babies being born. In seven women all the blastocysts implanted, in 18 women some implanted and some did not, which indicated that there was not a problem with the uterus, and in 23 women none of the blastocysts implanted, which indicated that either all the blastocysts were non-viable or that the uterus was not receptive.

When the babies were born, blood from the umbilical cord or swabs of cheek cells were taken and stored. The researchers used DNA

fingerprinting on these samples to match them with the DNA obtained from the blastocyst biopsies, thereby identifying which embryo grew into which baby. Then they used microarray to analyse the genetic message and find out which genes were expressed in the viable blastocysts. This work is still continuing, but already they have discovered that genes known to be involved cell adhesion, cell communication, cellular metabolic processes and response to stimuli – key processes involved in embryo implantation – are expressed in the viable blastocysts.

Dr Gayle Jones, a co-author and senior research scientist at the Monash Immunology and Stem Cell Laboratories, said: “We believe that it will be possible to refine our gene set to a smaller number of genes that is more highly predictive of a blastocyst’s viability and ability to develop to a term pregnancy when transferred to a receptive uterus than current selection criteria. The ability to select the single most viable embryo from within a cohort available for transfer will revolutionise the practice of IVF, not only improving pregnancy rates but eliminating multiple pregnancies and the attendant complications.”

The most important new findings from the research are:

- that up to 20 trophectoderm cells can be removed from a blastocyst without adversely affecting its viability and ability to implant;
- DNA fingerprinting is a very useful technique for discriminating between viable and non-viable blastocysts;
- trophectoderm cells from viable and non-viable blastocysts have different patterns of gene expression, which, when refined, could be used to select the single most viable embryo from a group for transfer.

Although more work needs to be undertaken before these findings

become applicable in the clinic, the researchers say that their work will also be useful for testing different treatments of embryos without the need to recruit large numbers of women to clinical trials, and DNA fingerprinting could be used to refine existing criteria for selecting embryos for implantation.

Dr Jones said; “Major improvements in IVF practice in the last decade have seen the introduction of better laboratory techniques that allow complete pre-implantation development to the blastocyst stage in vitro. One of the major stumbling blocks to worldwide acceptance of a single embryo transfer policy is the lack of highly predictive criteria to select the single most viable embryo within a cohort. The ability to use objective, measurable criteria rather than subjective observations, such as morphology, should improve the predictive value and provide sufficient confidence for clinicians to shift towards single embryo transfers for all patients without a concomitant drop in pregnancy rates. This would effectively reduce multiple pregnancies, which is a priority in the field of assisted reproductive medicine at present.”

Source: European Society for Human Reproduction and Embryology

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