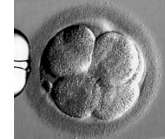


**In vitro fertilization (IVF)**  
**Preimplantation genetic diagnosis (PGD)**  
**Embryo Selection**

**In vitro fertilization**

- To get eggs
- Lupron is given for 2 weeks to shut down ovaries
  - Pregonal (human menopausal gonadotropin) or follicle stimulating hormone are given for 6 - 10 days to stimulate ovarian activity
  - hCG (human chorionic gonadotropin) is given to stimulate final egg maturation
  - A needle is used to remove eggs (5 - 15 usually obtained)
  - Progesterone is given after egg retrieval to enable the uterus to support pregnancy.

Eggs and sperm are mixed in a sterile petri dish  
 100,000 sperm per egg  
 Once a sperm has fertilized the egg, the embryo will begin to grow.  
 The embryo is allowed to grow to the 8 cell stage (about 3 days)



**IVF Statistics**

The first IVF baby was born in 1978 in England. To date about 20,000 IVF babies have been born.



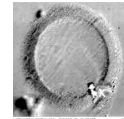
The success rate varies between 20 and 50% depending on the problems affecting a given couple.

Up to 4 embryos are implanted to maximize chances of success  
 25% of all IVF pregnancies are twins  
 2 - 3% are triplets.  
 Excess embryos are stored or destroyed  
 \$6,000 - \$7,000 per cycle

Abnormal embryos do not seem to mature to full term babies  
 Long-term risks are unknown, but so far IVF babies have done well.

**Preimplantation genetic diagnosis - PGD**

At the 8 cell stage, a hole is made in the protective coating of the embryo and one of the 8 cells is removed to serve as the genetic material on which genetic diagnosis is performed.



A cell can be removed from the embryo at the 8 cell stage and a normal human baby can (but doesn't always) result.  
 All genetic diagnostic tests must be performed on one cell!  
 one copy of each chromosome  
 All genetic diagnostic tests must be complete within 24 hrs so that the embryo can be implanted

**Uses of PGD**

PGD was introduced 14 years ago.  
 It allows carriers of fatal inherited diseases such as cystic fibrosis, sickle cell anemia, or Tay-Sachs to screen embryos before they are implanted thus avoiding abortion.  
 It also allows couples who have a high incidence of chromosomal abnormalities to select for abnormality free embryos.  
 Due to limited genetic material (1 cell) any given embryo can only be tested for a few traits/markers.

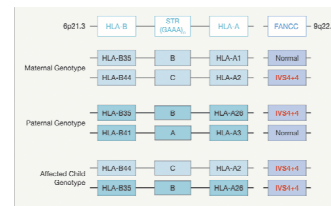
PGD has been performed in about 7,000 cases  
 1,000 healthy children have been born, most since 1999.

\$20,000 per cycle

Long-term effects on babies are unknown. So far, babies who are born have been healthy, although many embryos die.  
 In this case, the selected embryo benefited from IVF and PGD in that it was certain to not suffer FA.

**Genotypes of parents and affected child**

Note that the HLA genes are inherited together on chromosome 6



JAMA 285:3130

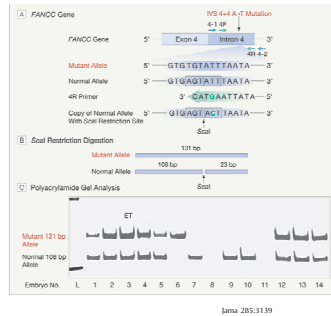
### PCR for FANCC *ivs4+4* mutation

Primer is constructed such that a Scal site will be generated in the normal but not the mutant allele.

Run PCR

Cut with Scal

Gel electrophoresis



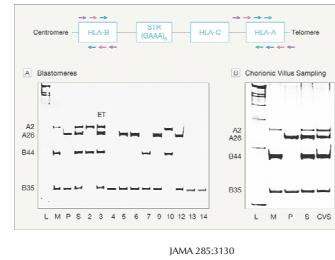
### PCR for HLA type

PCR was run using primers specific for

- HLA-A2
- HLA-A26
- HLA-B35
- HLA-B44

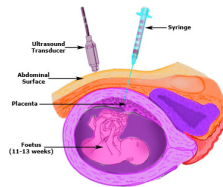
(if these are present, an appropriately sized product will be amplified)

Gel Electrophoresis



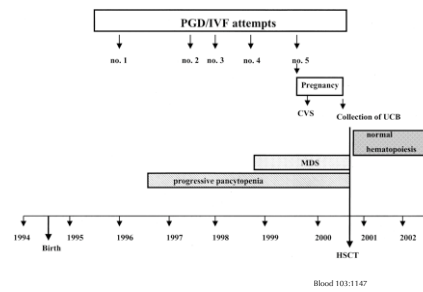
### Chorionic Villus Sampling (CVS)

Results were confirmed on material from chorionic villus sampling during pregnancy. A sample of placental material is taken and analyzed (between weeks 10 and 13).



Results from CVS also showed that the HLA-DR types matched as did blood type.

### Multiple Rounds of IVF and PGD were used



### Issues Discussed by Bioethics Committee

- Do the parents want another child?
- Equitable access. Procedure costs \$20,000 per round. How to be sure that everyone has same access to treatments.
- Which traits should be screened for?
- What if the first transplant with the cord blood fails? This leaves the parents with the choice of using an infant for a second BMT.