# CASE REPORT: P.G.D. for CF mutations and advanced maternal age.

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The PGD for cystic fibrosis is the most required due to the high frequency of carriers (1/25). CF is characterized by a wide diversity of mutations.
Besides, it is known that women more than 40 y.o. have an increased aneuploidy rate.

Our purpose is to comunicate the PGD performed in a normal couple aged 40 and 41 y.o.

Both are carriers of different mutations: (F508 and G542X.
The couple had a previous died affected child by CF.
They wanted to afford the PGD for CF and common aneuploidies.

Ovulation induction was performed with GnRh and Gonadotropins (Reliser+Gonal F from Serono™) beginning with Reliser 0.5 mg/d after ovulation and 0.25 mg/d from the day after menstruation until the HCG administration. Three ampulles of Gonal F 150 were administered during the first three days and one ampulle until day 11. Thirty four hours later of the administration of HCG 10.000 UI the OPK was performed transultrasonographycally.

Twenty one oocytes were recovered, 18 were MII and inyected with the husband's sperm. Fifteen oocytes were fertilised. Two cells from 6 good embryos wich reached the 8 cells at 72hs were biopsed. One cell was processed by PCR and the other was fixed for FISH study.

**Molecular analysis by PCR:**
The degenerated oligonucleotide primed DOP-PCR was used for whole genome amplification. The product was analysed in two complementary reactions for every mutation involved, one containing the primers for the normal DNA that does not amplify mutant DNA, the other the mutant primer that does not amplify normal DNA. Thus a normal embryo only will amplify in the normal reaction, a heterozygote in both reactions and a homozygous mutant only in the mutant reaction.

| | ARMS PCR PRODUCTS | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Normal | | Carrier ΔF508 | | Carrier G542X | | Affected | |
| Bp | 1 | 2 | 1 | 2 | 1 | 2 | 1 | 2 |
| 257 | | | | | - | | - | |
| 256 | | - | | - | | - | | - |
| | | | | | | | | |
| 160 | - | | - | | - | | - | |
| 157 | | | | - | | | | - |

Cell lysis was performed by freeze-thaw tecnique and Proteinase K treatment. For the DOP-PCR a degenerate primer was used.
Thermal cycling conditions were: 94ºC for 9min;eight cycles of 94ºC for 1min, 30ºC for 1,5min, 72ºC for 3min; 25 cycles of 94ºC for 1min, 62ºC for 1min, 72ºC for 1,5min and finally 72ºC for 8min.

Two microliters of the product originated were placed on two tubes, one of them contains an ARMS primer specific for normal (F508 and the mutant primer G542X sequence. The second tube contains an ARMS primer specific for normal G542X and the mutant primer (F508 sequence.

The cycling conditions for the two ARMS reactions were: 94ºC for 5min; 35 cycles of 94ºC for 2min, 60ºC for 2min, 72ºC for 2min and finally 72ºC for 10min.

| BCA | % Normal Segregation | Bp |
|---|---|---|
| DOP | CCG ACT CGA CAC TTC TAA TGA TGA TTA TGG GAG A | |
| ΔF508 | C: GAC CTT CAC TTC TAA TGA TGA TTA TGG GAG A<br>N: GTA TCT ATA TTC ATC ATA GGA AAC ACC AC<br>M: GTA TCT ATA TTC ATC ATA GGA AAC CCA TT | <br>160<br>157 |
| G542X | C: TAA AAT TTC AGC AAT GTT GTT TTT GAC C<br>N: ACT CAG TGT GAT TCC ACC TTC TAC<br>M: CAC TCA GTG TGA TTC CAC CTT CTC A | <br>256<br>257 |

C: common N: normal M: mulated

Electrophoresis of the products of the ARMS reactions was carried out in a 3% agarose gel containing ethidium bromide and visualized under UV light.

As Positive Controls were used limphocytes from the couple. As negative controls limphocytes

from non carriers.

We also carried out a control of non contamination using control blanks during all PCRs.

**FISH Analysis:**
For common aneuploidies we used Multivision PGT from Vysistm, which detects the chromosomes 13, 18, 21, X and Y by fluorescent signals green, acqua, red, blue and yelow, respectively.

The cells were fixed on slides with Carnoy I and then processed according the indications of Vysis. In brief, melting temperature 73ºC during 5 min., hybridization temperature 37ºC during 4 hours, rapid wash and antifade mounting.

**RESULTS**

| embryons | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| ΔF508 | - | - | + | No infor. | + | + |
| G542X | - | + | - | + | - | + |
| FISH | Nor. | Nor. | Nor. | Nor. | Nor. | +13 |
| 160 | No | Yes | Yes | No | Yes | Yes |

Embryos 1-6 Positives Controls

Embryos 2, 3 and 5 were transferred on day 6th.
The couple achieved a normal child (F508 Carrier).