

# The use of PGD in vitrified oocytes and embryos after repeat ovarian stimulation cycles in poor responder patients.

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## Background:

Poor responder patients have a reduced potential to produce an adequate number of oocytes and hence embryos. This low number of embryos available for biopsy will significantly reduce their chance for a successful preimplantation genetic diagnosis cycle (PGD). Repeat ovarian stimulation cycles combined with serial vitrification of oocytes and/or embryos obtained before the actual PGD could be an option to increase the chances for a healthy pregnancy in these poor responder patients.

## Aim:

This retrospective cohort study evaluates the efficacy of a PGD program in poor responder patients after repeat ovarian stimulation and serial vitrification of oocytes and/or embryos before the actual genetic analysis, this in combination with PGD on embryos obtained from an ultimate fresh ICSI cycle

## Results:

Tabel 1: Patient characteristics and laboratory outcomes		
Number of patients per indication	Total number	13
	Duchene Muscular Dystrophy	1
	Chromosomal translocation	1
	High sperm aneuploidy	1
	Cystic fibrosis	1
	SMN1	1
	X-linked Microtubular myopathy	1
	Repeated implantation failure	2
	Recurrent miscarriage	5
	Oocytes	Embryos
Number of cycles with vitrification of	6	18
Number of patients with vitrification of	3	10

Table 2: Laboratory and clinical outcomes		
	Oocytes	Embryos
Total number vitrified from repeat cycles	15	44
Survival after warming (number, %)	15 100%	44 100%
Number of oocytes/embryos obtained in ultimate fresh cycle	22	28
Total number of embryos available for PGD	20	72
Mean number of all embryos (fresh and warmed) per patient for PGD	7,2	
Number of patients with transfer of at least 1 healthy embryo (%) (patient with repeated failure of implantation had zero embryos for transfer after PGD)	12	92%
Mean number of embryos per transfer	2,1	
Number of patients with positive hCG test	9	
Percentage of patients with positive hCG test per transfer	75%	
Number of patients with healthy delivery	7	
Percentage of patients with healthy delivery per transfer	58,3%	

## Conclusions

This retrospective cohort study shows that poor responder patients in need of PGD can benefit from serial vitrification of oocytes and/or embryos after repeat ovarian stimulation cycles and hence improve their chance for a pregnancy and delivery of a healthy baby.

## Materials and methods

**Setting:** Private fertility treatment centre, Embryolab, Assisted Reproduction Unit, 173-175 Ethnikis Antistaseos 551 34 Kalamaria, Thessaloniki, Greece.

**Study design:** Retrospective cohort study over 30 months.

**Cycles-patients studied:** Thirteen poor responder patients entered our PGD program with the following indications: recurrent implantation failure, Cystic fibrosis, SMN1, X-linked Microtubular myopathy, Duchene Muscular Dystrophy, chromosomal translocation, high sperm aneuploidy or recurrent miscarriage. Following counseling, couples opted for serial vitrification of oocytes and/or embryos from repeat ovarian stimulation cycles followed by PGD.

**Patient's ovarian stimulation protocol** consisted of standard down regulation protocol or antagonist protocol. Patient stimulations were repeated because a limited number of embryos were obtained in the first cycle and all embryos were vitrified. One or two extra stimulation cycles were initiated to obtain a sufficient number of embryos for each patient.

**Oocyte collection** was carried out 36 hours post hCG administration. Fresh semen samples were prepared by density gradient and wash (Sage).

**ICSI** was done according to standard procedures.

**Check for fertilisation:** 18-22 hours post oocyte collection oocytes were checked for presence of 2 pronuclei (2PN). Fertilised oocytes were group cultured in 0,7ml droplets (Cleavage medium, Sage). Embryo quality was checked daily.

**Vitrification of oocytes and embryos:** Freshly collected oocytes and good quality day 2 embryos were vitrified using the Cryotech vitrification method and stored in liquid nitrogen.

**Warming of vitrified oocytes and embryos:** Oocytes and embryos were warmed according to Cryotech's guidelines.

**PGD:** Embryos were biopsied on day 3 of development and genetic testing was performed by FISH, PCR or CGH depending on the indication. Embryos were cultured individually in 50 µl droplets under oil until transfer (Cleavage medium, Sage). Embryo quality was checked daily.

**Embryo transfer:** Embryo(s) were transferred to the patient using a Wallace or Labotect soft catheter under by ultra sound guidance.

**Clinical pregnancy** is defined as the presence of a gestational sac with foetal heartbeat by ultrasound at 8-10 weeks after embryo transfer.

The IVF laboratory at Embryolab has **ISO 9001:2000 accreditation** (2007) and has been assessed in accordance to **ISO 15189-2007**.

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