

two hours post egg retrieval, embryos derived from in vitro matured injected oocytes were scored and reserved for embryo biopsy. Following embryo biopsy, cells were fixed using a modified Tarkowski technique. Fluorescent in situ hybridization (FISH) for chromosomes X,Y,13,15,16,17,18,21 and 22 was later carried out for aneuploidy assessment of the fixed cells.

RESULTS: Chromosomal assessment was performed on 43 embryos which developed from MII in vitro matured oocytes. After FISH analysis 23% were considered normal, 72% were abnormal and 5% provided no result. The rate of chromosomal anomalies was not significantly different in sibling embryos developing from oocytes which were mature at the time of cumulus removal (30% normal and 66% abnormal and 45 no result; n = 211)*. A large proportion of the abnormal embryos in the IVM group were either haploid/haploid mosaics (n=10) or triploid/triploid mosaics (n=8) accounting for a total of 58% of the abnormal embryos, whereas only 1.4% and 3.6% of the abnormal embryos in the sibling embryo group were triploid and haploid mosaics respectively. Additionally, embryo quality in terms of cell stage was statistically different in the IVM and sibling embryo sets of embryos; 25.5% and 10.6% being less than 5 cells on Day 3 respectively.**

CONCLUSION: The results of the present study indicate while a greater number of embryos derived from IVM oocytes are poor quality, those that are of appropriate cell stage on Day 3 (6-8cell) have aneuploidy rates that are not significantly different when compared to their sibling counterparts. However, this is a preliminary study and with further data collection, we may find a greater disparity in these two groups of embryos in terms of embryo quality and rates of aneuploidy. It may also be hypothesized that premature activation of an oocyte which has not completed or is in the process of completing meiosis I may cause incomplete separation of homologues, resulting in an increased rate of haploidy and triploidy in the resulting embryos.

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O-242

Preliminary Follow Up at 1 and 2 Years After PGD. L. Foix-L'helias, J. De Mouzon Sr., V. Kerbrat, P. Labrune, R. Frydman Sr., N. Achour-Frydman. Hopital Antoine Beclere Sce Pédiatrie, Clamart, France; Hopital Kremlin Bicetre Unite INSERM U569, Le Kremlin Bicetre, France; Hopital Antoine Beclere Sce Gyn Obs, Clamart, France; Hopital Antoine Beclere Sce Pediatric, Clamart, France; Hopital Antoine Beclere Sce Gyn-Obs, Clamart, France; Hopital Antoine Beclere Sce Biologie et Genetique Reproduction, Clamart, France.

OBJECTIVE: To investigate the physical and health status outcome at 1 and 2 year-old children born after PGD and compare them with children born after ICSI.

DESIGN: Prospective control study

MATERIALS AND METHODS: After each baby born after PGD, a control was chosen as the first next child conceived by ICSI controlling on maternal age (<35 and ≥ 35 years), number of babies (singleton or twin), and socioeconomical level (3 classes). Only the children presenting an important malformation at birth or very preterm (less than 33 weeks) were excluded for the comparison. All the children were examined at birth and at the age of 1 year by the Clamart neonatologist and a questionnaire was sent to the parents at 2 years. The 2 groups were compared with the usual tests (chisquare and Student's t test). The presented results concern the follow-up at 1 and 2 years.

RESULTS: In total, 24 PGD pregnancies (17 singleton and 7 twin), resulting in 31 children were selected, of which 4 were excluded of the comparison study, all singleton (1 Fallot tetralogy, 1 spina bifida and 2 very preterm (29 and 30 weeks). The control group consisted of 13 singleton and 7 twin pregnancies (27 children). At 2 years, the 2 groups consisted only of 18 and 17 children, because the others were still too young. The 2 groups had similar preterm labour (40.7% in each group). However, 7 fetuses were diagnosed intrauterine growth retardation in the PGD group, compared to 2 in the other, without reaching the statistical significance (25.9% vs 7.4%, p=0.14). At birth the sex ratio was low but similar for the two groups (0.59 vs 0.69, p=0.50). There was no difference in birthweight (2758 ± 599 vs. 2920 ± 553, p=0.31), size (47.2 ± 3.0 vs. 47.7 ± 2.4, p=0.54), Apgar score were similar. At 1 year, they had a similar, weight (9493 ± 960 vs.

9610 ± 1118, p=0.68), height (75.1 ± 2.8 vs. 76.3 ± 3.9, p=0.21), cranial circumference (46.2 ± 1.0 vs. 46.5 ± 1.1, p=0.21). Similar percentages of rhinopharyngitis (74.1% vs 81.5%), otitis (37.0% vs 59.3%), bronchiolitis (29.6% vs 40.7%). None was wearing glasses, or had an auditive correction, a kinesitherapy, a psychotherapy or was treated in a specialized centre. No developmental difficulty was observed: all the babies could sit, 87% could stand, 83% walked with a help and 28% by themselves. All were able to stretch out their hand towards an object, to give it. All of them were in good health. At 2 years, they had a similar weight (11899 ± 1017 vs. 11932 ± 1348, p=0.94), height (88.2 ± 5.0 vs. 85.1 ± 4.6, p=0.0.8), cranial circumference (48.6 ± 1.4 vs. 49.1 ± 1.4, p=0.40). There was no statistical difference in the percentages of rhinopharyngitis (83.3% vs. 50.0%), otitis (38.9% vs 50.0%), bronchiolitis (22.2% vs 26.7%). None was wearing glasses, or had an auditive correction, a kinesitherapy, a psychotherapy or was treated in a specialized centre. The number of sleeping hours was similar (13.61 ± 1.2 vs. 13.0 ± 1.5, p=0.23), as the percentage of babies with some difficulty to fall asleep (33.3% and 21.1%) and only 2 and 3 were awaking their parents frequently. There was no difference in feeding difficulty (5.6% and 10.5%). Only 6% of the parents in the PGD group and 29% in the control group had some fears for their child health and respectively 6% and 29% were thinking that he or she needed more care than a normal baby.

CONCLUSION: From this survey, even if numbers are still low, nothing was worrying in the babies health. There is still a need to increase the number of patients and to continue the survey for several years

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O-243

Preimplantation Genetic Diagnosis for Single Gene Disorders: Strategies and Results After Five Years' Experience. A. Biricik, F. Fiorentino, A. Nuccitelli, S. Kahraman, N. Vitale, M. Baldi. Embryogen - Centre for Preimplantation Genetic Diagnosis, ROME, Italy; "GENOMA" Molecular Genetics Laboratory, ROME, Italy; ART and Reproductive Genetics Unit, Istanbul Memorial Hospital, Istanbul, Turkey.

OBJECTIVE: Preimplantation Genetic Diagnosis (PGD) has demonstrated an established approach towards early diagnosis of genetic disorders, providing the opportunity for couples who have a known genetically transmittable disease to start a pregnancy with the knowledge that their child will be unaffected by the specifically tested disorder. PGD is a multidisciplinary procedure that requires combined expertise in reproductive medicine and molecular genetics. Although it is more than a decade since the first PGD for single gene disorders (SGD) was performed, the complexity of the approach has so far limited its clinical application. Thus, even if the numbers of centres performing PGD is increasing steadily, only few centres worldwide are offering PGD for SGD as a clinical service. This study reports the experience of our Centre, from 1999 to 2004, in PGD for SGD, describing strategies and overall clinical outcome of 240 PGD cycles performed for 23 different genetic conditions.

DESIGN: Over a 5 years period, a total of 167 couples were included in the PGD program. Referrals were received from IVF centres, geneticists and gynaecologists. Patients were counselled by a clinical geneticist, which first assessed the feasibility of carrying out the diagnosis of the genetic disease at single cell level. Finally the patients were referred to the IVF Centres to arrange the clinical aspects of the IVF treatment. The single gene defects investigated were autosomal dominant (15 cycles; 8 couples), autosomal recessive (142 cycles; 103 couples), or X linked disorders (19 cycles; 10 couples). A total of 64 cycles, for 46 couples, was also performed for SGD combined with HLA matching.

MATERIALS AND METHODS: A standard protocol for ovarian stimulation, oocyte retrieval, intracytoplasmic sperm injection (ICSI) and embryo biopsy was followed. The biopsied blastomeres were then transferred, contained in their sealed tubes, from the IVF centres to the PGD laboratory for genetic analysis. PCR reactions contained the external primers for the amplification of the gene regions involved by mutations, STR markers linked to these regions for ADO detection and those used for detection of aneuploidies, in patients with advanced reproductive age. Different strategies were used for mutation analysis, such as multiplex fluorescent PCR, linkage analysis or minisequencing, depending on the genetic disease investigated.

RESULTS: A total of 1859 embryos were analysed. PCR amplification was performed on 3271 blastomeres, obtaining a successful amplification in 3014 (92.1%) cells. Diagnosis was achieved for 1750 (94.1%) embryos, 409 of which were transferred to the patients in 202 cycles. Overall 60 pregnancies were established, 12 of which resulted biochemical, 6 spontaneously miscarried, two resulted ectopic and were terminated. The remaining pregnancies were confirmed to be unaffected by prenatal diagnosis; five pregnancies are still ongoing whereas the others went to term without complications, resulting in the birth of 35 healthy babies.

CONCLUSION: The clinical outcome of these cycles provides a further demonstration that PGD is an established clinical tool for assisted reproduction, complementing other similar experiences in the field. The rapid advances in molecular genetics are likely to stimulate further use of PGD and to encourage a substantial increasing of the range of genetic conditions for which PGD is offered.

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The Significance of Eliminating Chromosomally Abnormal Embryos in Recurrent Abortions Vs in Recurrent Implantation Failures. E. Kapetanakis, T. Damianaki, E. Levekki, N. Kouka, G. Maroulis. Center for Reproduction and Genetics, Athens, Greece; Democritus University of Thrace, Alexandroupolis, Greece.

OBJECTIVE: It is known that patients with recurrent abortions may have increased number of embryos with abnormal chromosomal constitution. Similarly, patients with recurrent implantation failure may have number of abnormal embryos that may affect Implantation Rate.(IR).

DESIGN: In this study we wanted to determine whether the elimination of chromosomally abnormal embryos is the common factor behind the problem of recurrent abortion and failure of implantation .We studied 3 groups of women for comparison A) Patient with recurrent abortion (group A) B) Patients with repeated IVF failure (group B) C) Patients with children who had sex-linked diseases in whom PGD was used to eliminate inheritance of sex-linked diseases and in whom the chromosomally abnormal embryos are not a factor in success (group C)

MATERIALS AND METHODS: We studied 153 women who were divided in 3 groups A) Women with at least 3 recurrent abortions (group A) (n=27) B)Women with repeated IVF failure (n=31) (group B),C) Women with children who had sex-linked diseases or didn't belong to group A or B (n=95) (group C). The women underwent IVF or ICSI procedures. The embryos were biopsied on day 3 and one blastomere from each was analyzed for X, Y,13,18,21 and 16 chromosomes using the fluorescent in situ - hybridisation (FISH) technique. Embryo transfer was performed the next day.

RESULTS: The age of the patients was not significantly different among groups (group A=34,2+-3.1, B=35,2+-2.9, C= 32.1+-2.9.What was significantly different was the % of normal embryos which was lower in group A and B than C. (AvsC p< 0.01, BvsC p< 0.01 .The elimination of the abnormal embryos improved the implantation rate (IR) and Pregnancy Rate (PR) in patients with recurrent abortions (group A vs group C NS) but in group B the PR was not improved and was much lower than in groups A and C. (p<0.01)

	Group A	Group B	Group C
Cases	27	31	95
Embryos examine with FISH	177	230	614
% Normal embryos	29,3	38,1	67
No of Embryos/ET	2,8	2,7	2,9
IR	12,0	2,9	16,0
PR	35,5	8,0	33,0
Abortion Rate	15	0	11,0

CONCLUSION: A) In both patients with recurrent abortion and repeated IVF failure of implantation there is a common underlying factor which is the significant increase in the development of abnormal embryos and B) while in

women with recurrent abortion the transfer of normal embryos brings about positive results the improvement with repeated IVF failure is less which suggests that in these women there are additional problems other than abnormalities in chromosomes X, Y 21,18,16 which may account for the decrease in IR.

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Impact of Embryo Quality on Developmental and Aneuploidy Rates After Blastomere Biopsy for PGD. H. F. Rodriguez, P. Hart, M. Abae. Center for Advanced Reproductive Endocrinology, Plantation, FL; LIFE Laboratories, Plantation, FL.

OBJECTIVE: To examine the impact of cleavage stage embryo quality on subsequent embryonic development and aneuploidy rates following blastomere biopsy.

DESIGN: Retrospective.

MATERIALS AND METHODS: Embryology data and PGD records for 189 embryos, which underwent day 3 blastomere biopsy for PGD of aneuploidy at our center were reviewed. Embryos were divided into euploid or aneuploid based on FISH analysis of 9 chromosomes (X, Y, 13, 15, 16, 17, 18, 21 and 22). The Mean patient's age for all cases in the study (n=22) was 37.0±5.6. Assessment of embryo morphology, blastomere biopsies and nuclei fixation were performed by the same embryologist. All biopsies were performed on day 3 and all FISH analysis were performed at Reprogenetics, N.J. Our laboratory uses a strict embryo morphology assessment system whereby embryos are evaluated systematically once every 24 hr period and assigned a quality grade ranging from A to E. Top quality embryos fall in categories A/B, Mid quality embryos fall in category C, Low quality embryos are assigned scores D/E. All data were compared using the Fisher's exact test.

RESULTS: Out of 189 embryos that were biopsied 88% had one blastomere removed, and 12% required an additional blastomere to be obtained. The distribution of 5 to 8 cell embryos at 60-62 hrs in culture was similar among those that were subsequently reported as aneuploid or euploid (94% vs. 93%, respectively). There was no difference in blastulation rate in embryos with one vs. those with two blastomeres removed. Overall, blastulation rates for Day-3 Top, Mid and Poor quality embryos were 65% (43/66), 56% (62/111), 17% (2/12), respectively. Accurate FISH results were available in 77% of all embryos biopsied. In this cohort, 72% (105/146) were determined aneuploid and 28% (41/146) euploid. Day 3 Top quality euploid embryos had higher blastulation rate, 83% (19/23), as compared to their aneuploid counter parts, 56% (18/32), (P=0.04). There was no significant difference in the blastulation rate of Mid and Low quality Day 3 euploid embryos when compared to their aneuploid cohorts. The table below summarizes aneuploidy / euploidy rates for Top, Mid and Low quality blastocysts.

Day 5 Morphology Assessment	Euploid	Aneuploid	P Value
	48%	52%	n.s.
Top Quality Blastocysts (Grades A/B)	(12/25)	(13/25)	
	36%	64%	0.01
Mid Quality Blastocysts (Grade C)	(16/45)	(29/45)	
	25%	75%	0.01
Low Quality Blastocysts (Grades D/E)	(4/16)	(12/16)	

CONCLUSION: In our initial experience, biopsy of one or two blastomeres does not appear to affect blastulation rate. However, day 3 embryo quality was associated with blastulation rate following biopsy. Based on FISH results, Top quality day 3 euploid embryos demonstrated greater blastulation potential. Approximately 50% of Top quality blastocysts were