

3.73%, respectively. Diploidy rates were also significantly increased in three patients from the infertile group. Overall, 12 out of 24 patients (50%) had a significant increase in sperm aneuploidy rates with 7 presenting an increased rate of XY gametes and 8 patients presenting an increased rate of 21 disomies. **Conclusion:** Our study shows that ICSI patients with a severe oligospermia have a higher rate of chromosomally abnormal gametes. These abnormalities with the use of ICSI could be transmitted to the offspring, with elevated risks of Klinefelter and trisomy 21 suggesting that genetic counselling should be offered to all patients entering an ICSI program.

P-520 Sperm aneuploidy screening by FISH in 310 infertile men

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Introduction: Seven percent of men face infertility problems in their lifetimes, but the aetiology is unknown in one out of three cases. One of the most common causes of male infertility is now considered to be of chromosomal aetiology, especially meiotic chromosome alterations. FISH screening of sperm makes it possible to determine whether the nucleus is euploid, diploid or disomic for any of the chromosomes studied.

Materials and methods: The FISH technique was performed on the sperm of 310 consecutive male patients who visited our clinic for infertility (none had produced a pregnancy). Their karyotypes were normal, their total sperm count was at least 0.5 million and they presented with no significant background factors such as orchitis, cryptorchidism, radiation therapy or chemotherapy. The FISH chromosome study done on the sperm was carried out using probes for chromosomes X, Y, 13, 18 and 21 (Vysis[®]). In 61 patients a study of meiosis in testicular biopsy was also carried out.

Results: The FISH results were normal in 262 patients (84.5%) and altered in 48 (15.5%). The alterations were disomies in 22 cases (45.8%); diploidies in 17 cases (35.4%); and disomies+diploidies in 9 cases (18.8%). The disomies (n=31) affected gonosomes in 19 cases (61.3%); chromosome 21 in 3 cases (9.7%); chromosome 18 in 2 cases (6.4%); and chromosome 13 in 4 cases (12.9%). More than one autosome was observed to be affected in 1 case (3.2%) and autosomes and gonosomes were affected in 2 cases (6.4%). The study of meiosis in testicular tissue (n=61) was normal in 19 cases (31.1%) and altered in 42 (68.9%). All the cases in which FISH was altered and a meiosis study was carried out had altered meiosis (n=19; 100%). In 23 cases (37.7%) the FISH results were normal and the meiosis study showed alterations.

Discussion: FISH screening of sperm provides information not available from standard semen analysis. Chromosomal alterations in sperm (disomies and/or diploidies) affect fertility and constitute an aetiological factor in infertility. The fact that more meiotic abnormalities were detected in testicular biopsy than in the FISH study may be explained by the limited number of probes used (only five), which means no information was provided on the 19 other chromosomes. In the meiosis study in testicular biopsy, however, all the chromosomes were studied. FISH screening of sperm is a useful technique in cases of infertility for unknown causes. In cases in which the FISH results are normal, it is also necessary to study the meiotic chromosomes in the testicle.

POSTER SESSION

Genetics: PGD

P-521 Preimplantation genetic diagnosis in Denmark

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Introduction: Preimplantation genetic diagnosis (PGD) is an alternative to prenatal diagnosis in case of risk of genetic disease in offspring. Whereas

prenatal diagnosis implies selective termination of pregnancy in case of a diseased foetus, PGD allows diagnosis before implantation. Liberal and free of cost access to PGD in Denmark was established in 1999. The purpose of the present study was to evaluate national activity and success rates and estimate the preferences in the population between prenatal diagnosis and PGD.

Materials and methods: National data was collected from all three PGD Centers in Denmark for the years 1999–2004 and from the national register for prenatal genetic diagnosis for the years 1997–2000.

Results: Data collection on PGD activity including November 2004 showed that the indication for PCR based PGD (46 couples) was cystic Fibrosis (11), Huntingtons Chorea (9), Myotonic Dystrofia (5), Fragile X (5) and other rare diseases (10). PGD using FISH (42 couples) was performed on indication of X-linked disease (15), translocations (26) and Di George syndrome (1).

A total of 227 PGD cycles were initiated during the period, resulting in 139 fresh embryo transfers (61%) and 13 frozen. A clinical pregnancy was detected in 22 cases (10% per cycle and 16% per embryo transfer).

A total of 88 couples during a 5-year period correspond to 18 couples a year, whereas the average annual number of prenatal diagnoses for a monogenic disease was 134.

Conclusions: The present data show a somewhat lower overall pregnancy rate than average reported European results (Sermon et al., 2005). This may be explained by a learning curve and/or by a rather high proportion of cases with translocations of which the reciprocal may yield few balanced or normal embryos. The national data elucidate that PGD activity seems rather low compared to prenatal diagnosis. This may be due to either patient preferences or to insufficient 'marketing'.

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P-522 Chromosomal abnormalities in embryos and spermatozoa from patients with recurrent hydatiform moles

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Introduction: Partial and complete hydatidiform moles are chromosomally abnormal pregnancies due to triploidy or diploidy, with the two sets of chromosomes belonging to the father. The objectives of this study were to analyse the incidence diploid and aneuploid spermatozoa in patients with recurrent hydatidiform moles as well as the percentage of chromosomal abnormalities in preimplantation embryos.

Materials and methods: This study included five sperm samples from patients with two previous molar pregnancies each. All of them were normozoospermic and had normal karyotypes. Chromosomal abnormalities were assessed by fluorescence 'in situ' hybridization (FISH) for chromosomes 13, 18, 21, X and Y and the incidence of disomy and diploidy was compared with a control group of five normozoospermic donors. Preimplantation genetic diagnosis (PGD) was performed in three of the couples and chromosomes 13, 16, 18, 21, 22, X and Y were analysed. FISH protocol was carried out as described by the probes manufacturers (Vysis Inc. Downers Grove, IL, USA). The percentage of abnormal embryos was compared with a control group of 25 patients undergoing PGD because of sex-linked diseases. Chi-square and Fisher's exact test were applied for statistical analysis.

Results: A significant increase in the incidence of chromosomal abnormalities was observed in three out of five sperm samples, one sample with higher incidence of disomy for sex chromosomes ($p < 0.0001$), a second one with higher incidence of diploidy ($p < 0.0001$) and a third one with an increase in both disomy for sex chromosomes ($p = 0.0485$) and diploid spermatozoa ($p < 0.0001$). PGD was attempted in the patient with increased incidence in disomic and diploid spermatozoa, but all the embryos were arrested on day 2, before the biopsy. In the two couples with normal FISH results on sperm, two PGD cycles were performed with 9 out of 10 abnormal embryos (90% versus 33.1% in the control group). The most relevant abnormality in the two cycles was mosaicism with 6 out 10 mosaic embryos (60% versus 7.2% in the control group).

Conclusions: Sperm chromosomal abnormalities could be related to the origin of recurrent hydatiform moles in some of the cases, impairing embryo quality. On the other hand, independently of the FISH results in spermatozoa, high incidence of abnormal embryos were observed in these couples and therefore PGD would help to diminish the risk of a further molar pregnancy.

P-523 Preimplantation diagnosis of the 8993T>G NARP mtDNA mutation

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Preimplantation genetic diagnosis (PGD) for women carrying pathogenic mitochondrial DNA (mtDNA) mutations is regarded as an alternative to prenatal diagnosis of mtDNA disorders for two reasons. First, it is feasible on single cells soon after fertilization (eight-cell stage). Second, owing to mitotic segregation of mtDNA species, a rapid genetic drift and markedly skewed segregation of mtDNA populations is expected to occur (bottleneck theory). However, the predictive value of a PGD requires that the proportion of mutant mtDNA species diagnosed in the biopsied cells of the embryo (blastomeres) be an accurate indication of the mutant load in the remaining embryo.

In order to address the question of whether a given blastomere is representative of the status of the whole embryo, individual blastomeres from fifteen 4-day embryos were collected. A hypervariable region 2 (HV2) consisting of a homopolymeric tract of cytosines and exhibiting length polymorphism was PCR amplified. Striking variations in level of HV2 heteroplasmy were found among embryos, but, differences in blastomeres derived from a single embryo were limited ranging from 0 to 19% (mean 7%).

Considering these data, we performed PGD for the neurogenic ataxia retinitis pigmentosa (NARP) mtDNA mutation (8993T>G) in the carrier mother of an affected child. Three embryos were analyzed, 1/3 was found to carry 100% of mutated species in the two blastomeres tested while none (2/3) of the other embryos showed detectable amount of mutant mtDNA. The two mutation-free embryos were transferred and resulted in a normal pregnancy and in the birth of a healthy child. Why the 8993T>G NARP mutation in the three embryos of the carrier mother reported here segregated in a markedly skewed manner, compared to the HV2 mtDNA length polymorphism, is still questionable. However, these preliminary data suggest that the level of heteroplasmy from a single blastomere is representative of the status of the whole embryo. In conclusion, PGD for a pathogenic mtDNA mutation is feasible but should be approached with caution, as transmission of pathogenic mutations may markedly differ from that of mtDNA polymorphisms.

P-524 PGD: how to destroy a scientific discipline. The Italian experience

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Introduction: Ten months after the introduction of a law regulating assisted reproductive technology in Italy, the influence of the imposed restrictions are becoming clear. One of the most sharp criticisms regards the prohibition of performing PGD in the name of 'protection of the embryo'. This is especially contradictory when considering that Italy, since 26 years, has a liberal abortion law. In this reality, there is a total protection over early preimplantation embryos while fetuses are totally unprotected. Disease-related indications to PGD are widely considered not only as ethically accepted interventions, but also as clinically recommendable in the framework of prevention. Its banning from medical practice exposes couples at reproductive risk to repeated terminations of pregnancy as the only alternative to affected concepti. In the present study, an estimation of the reproductive chances for couples with PGD indications has been attempted, by comparing the performance following the restrictions imposed by the legislation with that obtained before the current regulations. It is important to keep in mind that the new IVF regulation imposes

not to generate more than 3 embryos, implying that only 3 oocytes can be inseminated. In addition, the analysis of the first polar body (PB1) is the only form of PGD admitted.

Materials and methods: While real data were available in the case of PGD for aneuploidy, for carriers of single gene disorders no couple accepted to rely upon PB1 biopsy, preferring to cancel the treatment or to do PGD in countries where it is permitted. In this case, a simulation was done by relating the results obtained in our PGD program to a hypothetical figure of 100 metaphase II oocytes. This was calculated for PB1 biopsy, first and second polar body (PBs) biopsy, embryo biopsy (EB) and conventional treatment (CT).

Results: In the case of single-gene disorders, taking into consideration the events of no result, allele drop-out, recombination at meiosis and the implantation rate per oocyte, the number of infants born would be 1.5 for PB1, 4.03 for both PBs, 7.20 for EB and 6.98 for CT. In CT, the patient is exposed to 9.58 interventions of prenatal diagnosis and 2.50 interventions of therapeutic abortion. Based on the mean number of collected oocytes, the number of cycles necessary for a healthy infant in relation to maternal age is:

Maternal age (years)	≤29	30–34	35–37	38
No. of cycles – PB1	4.54	5.88	7.14	8.33
No. of cycles – CT (three inseminated oocytes)	2.38	2.56	2.86	2.94
No. of cycles – CT (inseminated all oocytes)	1.00	1.26	1.51	1.85

In the case of aneuploidy, the observed implantation rates are 15% for PB1, 18% for both PBs, 24.7% for EB and 10.8% for CT; the proportion of aborted gestational sacs is 21, 20, 9.1 and 37.5%, respectively. In CT, the patient undergoes 3.78 interventions of prenatal diagnosis, 0.2 interventions of therapeutic abortion and 2.27 interventions of curettage for spontaneous abortion. The restriction to three oocytes for insemination worsens even more the performance, increasing the number of cycles necessary for a healthy infant (1.4-fold for PB1 and 1.9-fold for CT).

Conclusions: The analysis of PB1 or a CT in patients with PGD indications are much less efficient compared to EB. The disadvantage is especially severe when the insemination is restricted to three oocytes, forcing the patient to undergo a higher number of cycles to have an infant born. In this context, young women are more disadvantaged compared to older patients. In addition, the numerous interventions of prenatal diagnosis and of therapeutic and spontaneous abortions consequent to CT, greatly threaten the physical and psychological integrity of the patient.

P-525 Myotonic dystrophy: does it affect ovarian follicular status and responsiveness to controlled ovarian hyperstimulation?

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Objective: Myotonic dystrophy (MD) is an autosomal dominant disease characterized by myotonia, muscular dystrophy, cataracts, cardiac abnormalities and hypogonadism. Indeed, in men, MD may cause testicular atrophy and oligospermia due to tubular primitive lesions (Vazquez et al., *J. Endocrinol. Invest.* 1990). Yet, in women, the relationship between MD and infertility remains unclear. Some investigators have associated MD with an increased risk of premature ovarian defects (Ulloa-Aguirre et al., *Obstet. Gynecol.* 1981), whereas others failed to find a relationship between MD and fertility (Roy et al., *Genus* 1989). These uncertainties spurred us to investigate the ovarian follicular status and response to COH in women suffering from MD and entering our preimplantation genetic diagnosis (PGD) program. For this, we elected to compare them with cystic fibrosis (CF) carriers, given that CF has not been hitherto shown to affect ovarian fertility.

Materials and methods: We compared 44 cycles of PGD for MD with 33 cycles of PGD for CF. On cycle day 3, all women underwent serum FSH, E2 and inhibin B measurements and antral follicle counts (AFC) by ultrasound. Subsequently, COH was performed with a combination of GnRH agonist and exogenous gonadotropins in a long protocol. When at least five mature follicles

reached 16 mm in diameter, hCG was administered. All oocytes obtained were inseminated by ICSI (day 0). On day 3, two blastomeres were removed from each embryo for PGD. Embryo transfers were performed on day 4.

Results: MD and CF groups were similar with regard to women's ages (30.6±0.4 years versus 31.7±0.5 years), day 3 FSH (6.5±0.3 mIU/ml versus 6.9±0.2 mIU/ml), E2 (39.0±3.60 pg/ml versus 52.1±6.0 pg/ml), and inhibin B (62.4±4.0 pg/ml versus 74.4±6.5 pg/ml) levels, and AFC (14.5±1.0 versus 16.5±1.7), respectively. They were also comparable with regard to cycle cancellation rate for poor ovarian response (23% versus 18%), as well as to the number of 16 mm follicles on the day of hCG (9.0±0.4 versus 8.0±0.6), oocytes retrieved (12.6±0.9 versus 12.9±1.2), embryos obtained (7.2±0.6 versus 6.6±0.7), the prevalence of top quality embryos (39% versus 41%) and implantation rates (13% versus 10%), respectively.

Conclusions: (1) The present results indicate that the ovarian follicular status, responsiveness to COH, and implantation rates were not hindered in women affected by MD as compared to controls. The mechanisms that could explain why MD cripples testicular function and spares the ovarian potential remain to be determined. (2) These reassuring data supports the feasibility of PGD in women with MD.

P-526 Strategies and outcomes of over 200 cycles of preimplantation genetic diagnosis for single gene disorders

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Introduction: Couples at high risk of passing on a serious genetic condition to their offspring have the opportunity to use preimplantation genetic diagnosis (PGD) to diagnose a specific genetic disease on embryos obtained through in-vitro fertilization (IVF) before a clinical pregnancy has been established. This article reports the experience of our Centre, from 1999 to 2004, in Preimplantation Genetic Diagnosis (PGD) for single gene disorders (SGD), describing strategies and overall outcome data of 222 PGD cycles performed on embryos of 158 couples for 22 different genetic conditions.

Materials and methods: The single gene defects investigated were autosomal dominant (n=14), autosomal recessive (n=133), or X-linked disorders (n=19). Fifty-five cycles were performed for SGD combined with HLA matching. Mutation analysis of gene regions affected by mutations was carried out using both minisequencing method or multiplex fluorescent PCR.

Results: A total of 1683 embryos were analysed. PCR amplification was performed on 2955 blastomeres, obtaining a successful amplification in 2723 (92.1%) cells. Diagnosis was achieved for 1580 (93.9%) embryos, 380 of which were transferred back to the patients in 186 cycles. Overall 54 pregnancies were established (29.0% per transfer), 10 of which resulted biochemical, six spontaneously miscarried, two resulted ectopic and were terminated. All ongoing pregnancies were confirmed to be unaffected and went to term without complications, resulting in the birth of 33 healthy babies.

Conclusions: The present results complement other similar experiences in the field and confirm the feasibility of PGD when applied in the context of a preventive genetic service.

P-527 Sperm chromosomal abnormalities and PGD: relationship with sperm parameters

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Introduction: Fluorescence in situ hybridization (FISH) in spermatozoa has revealed an increased incidence of chromosomal abnormalities in infertile men, mainly related to oligozoospermia. The aim of this study was to assess the incidence of chromosomal abnormalities in preimplantation embryos from infertile couples with an increased incidence of chromosomal abnormalities in spermatozoa and their relationship with sperm parameters. Reproductive outcome after preimplantation genetic diagnosis (PGD) was also evaluated.

Materials and methods: FISH studies in spermatozoa to assess the incidence of diploidy and disomy for chromosomes 13, 18, 21, X and Y were indicated to severe oligoasthenoteratozoospermic (OAT) and teratozoospermic patients.

Individual FISH results were compared with a control group of five normozoospermic donors, and considered as abnormal when significant increases for disomy and/or diploidy rates were observed. Disomy for sex chromosomes was the most frequent abnormality observed in these patients. Subsequently, PGD was performed in a total of 41 couples with normal blood karyotypes and abnormal FISH results in sperm and results were evaluated according to sperm parameters (29 cycles in OAT patients and 12 cycles in teratozoospermic patients). PGD outcome was compared with a control group of fertile women undergoing PGD because of sex-linked diseases (25 cycles). In all cases the women's age was <36 years and chromosomes 13, 16, 18, 21, 22, X and Y were evaluated by FISH following the standard protocol as described by Vysis Inc. (Downers Grove, IL, USA). Mosaicism was estimated as discordant results when two blastomeres from the same embryo were analysed. Fisher's test was employed for statistical comparisons (p<0.05).

Results: A total of 135 embryos were analysed from OAT patients and 60 embryos from teratozoospermic patients. The percentage of abnormal embryos was 61.5% in the OAT group and 48.3% in teratozoospermic patients, showing significant differences with the control group (33.1%) in both cases (p<0.0001 and p=0.04, respectively). Mosaicism was also significantly increased in the two groups (44.3%, p<0.0001, and 20.6%, p=0.0486, respectively) compared to controls (7.2%). The incidence of aneuploid embryos for sex chromosomes was only significantly increased in the OAT group compared to controls (16.5% versus 7.7%, p=0.0277), without statistical differences in teratozoospermic patients (13.3%). Pregnancy outcome was similar in the two study groups (52.2 and 54.5%) and the control group (50.0%), with comparable implantation (42.1, 31.8 and 33.1%) and miscarriage rates (8.3, 16.7 and 8.3%).

Conclusions: Patients with abnormal FISH results in spermatozoa showed an increased incidence of chromosomally abnormal embryos compared to controls, mainly due to high mosaicism rates. There was a correlation with the increased incidence of abnormalities for sex chromosomes in both, sperm samples and embryos. Therefore PGD with the selection of euploid embryos would improve the reproductive outcome of these couples in terms of ongoing pregnancy and live-births with lower risk of chromosomopathy.

POSTER SESSION

Genetics: PGS

P-528 Intraindividual recurrence of aneusomy rate in polar body

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Introduction: Our aim was to evaluate the intraindividual variation of the incidence of human first polar body – and thus of metaphase II oocytes – aneusomies. We compared the first polar body aneusomy rate in the course of two successive ovarian stimulations. The stability of this rate for each patient would allow to use the first analysis as a prognosis tool, and to strategically adapt the following therapies.

Materials and methods: The study concerned 13 women who all underwent a second ICSI attempt with preconceptional diagnosis (PCD), performed for maternal age or implantation failure, after an unsuccessful first attempt with PCD (no pregnancy). A total of 105 first polar bodies were biopsied, with a Nikon inverted microscope equipped with a Hamilton laser system. Each polar body was hybridized with a MultiVysion™ PGT multicolour probe panel (ABOTT) for chromosomes 13, 16, 18, 21, 22, labelled respectively with SpectrumRed, Aqua Blue, Green and Gold fluorochromes. After a 4-h hybridization the slides were washed and observed with an Olympus BX 60 fluorescent microscope. Polar bodies were analysed and judged according to the number of spots for each given chromosome.

Results: Forty-eight polar bodies were analysed for the 13 patients during the first cycle, among them 23 were abnormal (48%), 57 oocytes were analysed during the second attempt of the same 13 patients; among them 34 were abnormal (59%). Percentage of abnormal oocytes in an oocyte cohort varied