differences were found within any of these subgroups in terms of pregnancy or implantation rates.

Subgroups A and B showed no differences in pregnancy (58% vs. 58%) or implantation rates (29% vs. 27%) when compared regardless of application of the technique. Subgroup C showed lower pregnancy (12% vs. 58%, p<0.001) and implantation rates (8% vs. 27%, p=0.011) independently of undergoing AHA and lysed cell removal when compared with subgroups A and B.

Conclusion: In our study, pregnancy and implantation rates in thawed embryo transfers seem to correlate with the survival of the embryo and the presence of lysed cells do not seem to inferfere in the correct development of the embryos. In our study, AHA and lysed cell removal did not alter the pregnancy or implantation rates of thawed embryos. To assess the real impact of this technique higher number of patients is needed in order to have a relievable statistical power.

O-104 The influence of a restrictive selection of the embryos to be frozen and the day of thawing on the results of our cryopreservation programme

N. Ortiz-Piñate, G. Pérez-Bermejo, C. Hernández, A. Rubio, O. Collado, I. Bruna, F. Prados
Hospital de Madrid-Montepríncipe, Unidad de Reproducción, Boadilla del Monte, Spain

Introduction: A good Frozen Embryo Transfer (FET) programme is compulsory prior to an effective reduction in the number of fresh embryos replaced without compromising the pregnancy rate per oocyte retrieval. Our purpose was to determine the effect of a strictly morphological selection of the embryos to be frozen on the success of our cryopreservation programme. We also compared the FET results obtained in cycles in which the embryos were thawed the day before the transfer with those of embryos thawed a few hours before the transfer.

Material and methods: The human embryos included in this study were frozen and thawed for transfer from 2001 to 2005 in our centre. Embryos were generated from 256 homologous IVF cycles. Two periods of time were compared. Cycles performed before 2004 (Group I) corresponded to cryopreservations in which all supernumerary multicellular embryos were frozen. During the second period (Group II) embryos were strictly selected on the basis of their morphological appearance prior to cryopreservation. Embryos were thawed one day before the transfer (49 FET) or the same day (107 FET). All embryos in this study were cryopreserved using a slow-freezing protocol with 1,2-propanediol as cryoprotectant.

Results: Group I: 57.0% of the 1062 embryos (2PN) obtained in the 150 oocyte retrievals were frozen, while in the 106 retrievals of Group II we froze 41.5% of the 884 embryos produced. 356 embryos (Group I) were thawed in 115 cycles and 210 embryos were transferred in 101 FET resulting in to 25 ongoing pregnancies (24.8% per FET). In 73 cycles from the Group II, 211 embryos were thawed and 127 were transferred in 65 FET, 22 of which resulted in ongoing pregnancies (33.8% per FET). The implantation rate of Group II was significantly higher (26.8%) than that of Group I (15.7%). The cumulative ongoing pregnancy rate per oocyte retrieval was 62.0% for Group I and 59.4% for Group II. For the second part of this study, 49 FET were performed 1 day after thawing 143 embryos, of which 86.7% survived. The implantation rate was 16.2% and the ongoing pregnancy rate per transfer was 24.5%; rising to 26.8% for the 41 transfers in which the embryos were frozen 2 days after the oocyte retrieval. These results were compared with those of the 107 cycles in which 334 embryos thawed the same day of the transfer. In this case, the survival rate was 74.6%, the implantation rate was 22.8% and the ongoing pregnancy rate per transfer was 32.7%. This pregnancy rate rose to 37.0% for the 81 FET in which the embryos were frozen on day 3 after retrieval.

Conclusion: A restrictive strategy for the selection of embryos prior to its freezing (Group II) lead to a higher ongoing pregnancy rate per FET and significantly higher implantation rate than that obtained with a permissive strategy (Group I). However, the cumulative ongoing pregnancy rate per oocyte retrieval was slightly lower in Group II. The careful selection of embryos due to be frozen reduces the number of transfers that a patient has to go though without compromising her likelihood of pregnancy per oocyte pick-up. The success rate for embryos thawed the day of the FET (mainly day 3 frozen embryos) was higher than that of embryos incubated 1 day prior to transfer (day 2 frozen embryos highly represented). Our results suggest that the best strategy is to select the embryos for freezing on day 3 and to carry out the thawing on the day scheduled for the frozen embryo transfer.

FREE COMMUNICATION

Session 29 – Genetics—PGD

Tuesday 20 June 2006 10:00–11:30

O-105 Development of an improved preimplantation genetic diagnosis test for fragile X syndrome and clinical application

P. Burlet[1], N. Frydman[2], N. Gigarel[1], R. Gesny[1], B. Aral[1], G. Tachdjian[2], V. Kerbrat[3], E. Feyereisen[3], J.P. Bonnefont[1], R. Frydman[3], A. Munnich[1], J. Steffann[1]
[1]Hôpital Necker, Genetique, Paris, France; [2]Hôpital Beclere, Biologie de la reproduction, Clamart, France; [3]Hôpital Beclere, Gynecologie obstetrique, Clamart, France

Introduction: Fragile X syndrome (FXS) is the most common monogenic cause of mental retardation. It is caused by mutations in the Fragile X Mental Retardation-1 gene (FMR1), more than 95% of which involve hyperexpansion and hypermethylation of a polymorphic CGG trinucleotide repeat in the 5′ untranslated region of the gene. Both males and females can be affected, leading to a high recurrence risk for this disorder, such that 'at-risk' couples often ask for preimplantation genetic diagnosis (PGD). PGD for FXS remains difficult. First, technical difficulties have been reported. Two alternative methods are used for single blastomere analysis, i.e. detection of the non expanded CGG repeat allele, or use of linked polymorphic markers. Direct detection of the normal allele is difficult due to the lack of PCR sensitivity despite specific protocols (the expansion is refractory to PCR due to the high GC content of the repeat and surrounding sequences) and leads to a risk of allele drop-out (ADO). In addition, this approach is suitable for only 63% of couples, the heterozygosity of the repeat in the normal population. The main drawback of the indirect method is the risk of recombination and/or ADO which may lead to misdiagnosis, and the non-informativity of the markers which required to establishing a diagnostic test for each family. A second limitation to PGD for FXS is that women carrying premutation are at increased risk for premature ovarian failure. A reduced ovarian response to stimulation protocols often results in few embryos available to test and thus to transfer. Diagnosing each embryo is therefore a true challenge.

Methods: Recently, Multiple Displacement Amplification (MDA) has been reported to yield large amount of DNA from single-cells. In order to increase reliability and sensitivity of the FXS diagnosis, we developed a combined analysis of polymorphic markers (i.e. DXS548, DXS1215, FRAXAC1, DXS998) with the study of the non-expanded CGG allele, and the amelogenin sequence for gender determination, using MDA as a first step.

Results: Single-cell amplification efficiency was first assessed on single lymphocytes for both normal CGG alleles and the four linked microsatellite markers. Amplification rate ranged from 88 to 95% with an ADO rate comprised between 5 and 34%. Using this test, 5 PGD cycles were carried out for five couples, and 53 blastomeres (26 embryos) were analyzed after preliminary MDA. Amplification rate was increased by this technique from 41 to 64%, so that embryos with no results were rarer (19% vs. 45% without MDA). Reliability of the test was considerably improved by combining both direct and indirect analysis of the cells. The use of several polymorphic markers indeed provides independent diagnostic confirmation. Particularly, the risk of misdiagnosis due to ADO at one locus was avoided by extrapolating the information obtained from other loci, and the use of the amelogenin sequence, a method of sexing the embryos, enabled distinction between ADO and hemizygosity. Furthermore, in cases of fully expanded alleles too large to be amplified by PCR, this test gives an internal amplification control. It can also detect contaminations by showing exogenous alleles in linkage analysis and give some information on the ploïdy of the embryos.

Conclusions: Embryonic transfers were carried out in all PGD cycles. One biochemical and one clinical pregnancy resulted, and a healthy child was

born. MDA is a powerful tool improving both sensitivity and reliability of FXS PGD. This single diagnosis procedure could be suitable to most patients carrying FXS.

O-106 Comparison of the results of preimplantation genetic diagnosis for single gene disorders combined with or without HLA typing

G. Karlikaya[1], H. Karadayi[1], S. Sertyel[1], S. Saglam[1], B. Umay[1], S. Kahraman[1], F. Fiorentino[2]
[1]Istanbul Memorial Hospital, Reproductive Endocrinology ART & Genetics unit, Sisli Istanbul, Turkey; [2]Laboratorio Genoma, Genetics, Rome, Italy

Introduction: Preimplantation genetic diagnosis (PGD) for single gene disorders (SGD) has become an alternative method to prevent the birth of an affected child. Also, many couples are now demanding PGD testing for the curative therapy of their affected children suffering from haematopoietic disorders by selecting HLA-compatible healthy embryos. However, several patient specific factors like advanced maternal age and diminished ovarian reserve as well as low chance of finding HLA compatible healthy embryos limit the successful outcome. In this study, we evaluate how the rate of HLA compatible embryos could affect the outcome.
Materials and methods: This study was conducted at Istanbul Memorial hospital IVF and Genetics department between 2002 and 2006. 35 couples with 42 cycles in SGD group and 61 couples with 99 cycles in HLA±SGD group were evaluated. After using standard controlled ovarian hyperstimulation protocols, ICSI was performed in all cases in order to eliminate the risk of contamination. On third day of embryo development, one or two blastomeres were biopsied to analyze the single gene defect and/or HLA compatibility. Two-round PCR was performed after which minisequencing method was performed to analyze the mutation of interest and STR analysis was performed to determine the HLA haplotypes. Selected embryos were transferred on fourth or fifth day. Mann-Whitney U test, Student *t*-test and chi-square test were applied to compare the results, where appropriate.
Results: In comparison of SGD and HLA±SGD group, no significant difference was found between two groups in terms of patient's age (32.5±4.8 vs. 32.5±5.3), number of mature oocytes (11.02±6.7 vs. 12.1±7.1) and number of biopsied embryos (8.5±4.4 vs. 9.2±5.6) ($p<0.05$). While 69.7% of the embryos were found to be suitable for transfer in SGD group after mutation analysis, only 11.2% of the embryos were suitable for transfer in HLA±SGD group since HLA compatibility of the embryos was also considered ($p<0.001$). In SGD group, only 2 out of 42 (4.8%) cycles were cancelled due to the lack of healthy embryos, while in HLA±SGD group, 37 out of 99 (37.4%) cycles were cancelled due to the lack of healthy and HLA compatible embryos ($p<0.001$). Although the pregnancy rates in transfer cycles of the two groups were similar (47.5% vs. 35.5%, $p>0.05$), the pregnancy rates were higher in SGD group according to the cycles initiated (45.2% vs. 22.2%, $p<0.001$). In SGD group, 6 cases ended in early spontaneous miscarriage, 5 singleton pregnancies are ongoing, 10 healthy babies were born (5 singleton, 2 twins, 1 triplet). In HLA±SGD group, 8 cases ended in early spontaneous miscarriage and 1 ectopic pregnancy was diagnosed, 9 pregnancies are ongoing (8 twins, 1 singleton), 5 healthy babies were born (3 singleton, 1 twin). In two cases, hematopoietic stem cells (HSC) obtained from umbilical cord blood were successfully transplanted.
Conclusion: Advances in IVF and molecular genetics not only improve the outcome in infertility cases but also provide to have a healthy child for the couples who are carriers of a single gene disorder and give an opportunity to have an HLA compatible sibling for the curative therapy of their affected child as well. However, low probability of finding both HLA compatible and healthy embryos for transfer limits the successful clinical outcome. Before starting each cycle for SGD or HLA±SGD, this information should be discussed in detail with the couples. More importantly, these couples, before planning their family, should be informed by physicians about these options.

O-107 Pregnancy and birth outcomes following preimplantation genetic diagnosis with blastocyst biopsy compared with day 3 biopsy

S. McArthur, J.T. Marshall, D. Leigh, M. Traversa, A. Gee, R.P.S. Jansen, K. de Boer
Sydney IVF, Preimplantation Genetic Diagnosis, Sydney, Australia

Introduction: For preimplantation genetic diagnosis analysis, embryos were cultured to the blastocyst stage of development on day 5 or 6 and blastocyst biopsy—removal of trophoblast cells—used as the standard method of embryo biopsy, for both polymerase chain reaction (PCR) (where testing protocols have been developed for over 80 different diseases) and for fluorescent in situ hybridization (FISH) analysis (for chromosome enumeration and translocation testing). Here we report on pregnancy and birth outcomes following more than 100 births after PGD using blastocyst biopsy.
Materials and methods: Embryos were cultured using Sydney IVF stage-specific medium (Cook IVF). Cells for analysis were obtained via trophectoderm biopsy using a Hamilton-Thorne Zilos Tk Laser or FERTILASE near-infra-red 1380 nm diode laser, attached to an Olympus IX-70 inverted microscope. From December 2000 through December 2005, 671 PGD cycles were undertaken utilizing blastocyst biopsy. These cycles were analyzed for clinical pregnancy data and compared with results achieved using day 3 embryo biopsy in our PGD program. Birth data and medical follow up were compared for IVF embryos transferred at the stage of blastocyst (428 pregnancies) and a PGD blastocyst biopsy cohort (87 pregnancies).
Results: To date there has been 107 births following blastocyst biopsy, including 17 from embryos frozen and thawed after blastocyst biopsy and PGD analysis compared with 92 live births following day 3 embryo biopsy. There are an additional 52 ongoing pregnancies following blastocyst biopsy. Comparing IVF embryos that reach the stage of blastocyst development, biopsy of trophectoderm for PGD had no apparent detrimental effect on gestational age (mean gestational age of 273 days in both cohorts), birth weight (mean birth weight biopsied 3.38 kg vs. mean birth weight not biopsied 3.33 kg) or infant development (Table I).

Table I.

	Day 3 biopsy 1996–May 2004	Day 5 biopsy 2003–Dec 2005
Clin. Preg per cycle	76/347(22%)	131/671 (20%)
Clin. Preg per transfer	76/261(29%)	131/317 (41%)
Implantation rate	101/359 (28%)	136/347 (39%)
Average no. embryos transferred	1.4 359/261	1.1 347/317
Average age	34	36

Conclusion: PGD with blastocyst biopsy provides patients with improved opportunities for preimplantation genetic diagnosis and pregnancies compared with day 3 embryo biopsy, without detrimental effect on resulting live births and in most cases with normal subsequent follow-up. The benefits include (1) utilizing blastocyst culture and elective single embryo transfer (eSET) to reduce the risk of multiple pregnancies, (2) a higher implantation rate per embryo transferred, (3) a higher clinical pregnancy rate, (4) a lower miscarriage rate and (5) a higher take home baby rate at normal birth weight.

O-108 Multiple displacement amplification as a method of whole genome amplification for preimplantation genetic diagnosis

S. Glentis[1], S. SenGupta[1], A. Thornhill[2], I. Craft[2], N. Carter[3], L. Rickman[3], C.J. Harper[1]
[1]University College London, Obstetrics and Gynaecology, London, UK; [2]London Fertility Centre, Human Genetics, London, UK; [3]The Welcome Trust Sanger Institute, Molecular Cytogenetics, Cambridge, UK

Introduction: Whole genome amplification (WGA) is a procedure aimed at achieving robust amplification of the entire genome from as little as 10 ng of genomic DNA. WGA has been used in many different areas of biology and medicine, including genetic tests in samples with a limited amount of DNA present, such as preimplantation genetic diagnosis (PGD). Different WGA methods have been developed that can largely be separated into two groups: Polymerase chain reaction (PCR)-based methods, such as degenerate oligonucleotide primed PCR (DOP-PCR) and non-PCR-based methods like multiple

displacement amplification (MDA). MDA is a relatively new non-PCR technique that utilizes phi29 DNA polymerase to amplify the entire genome in a few hours. The purpose of this study was to evaluate MDA using three different post-amplification methods (fluorescent PCR, comparative genomic hybridization (CGH) and microarrays) when the starting DNA template came from a single cell in order to achieve accurate results for PGD.

Materials and methods: Fresh buccal cells were used as a source of single cell DNA. The single cell lysis was done using standard Proteinase K or alkaline lysis protocols, according to the WGA technique used. Control genomic DNA was obtained by conventional extraction methods from peripheral blood samples taken from the same subjects as the buccal cells. DNA from single cells was amplified either by MDA (Repli-g kit, Qiagen, UK) or by DOP (DOP primer, Oswel, UK) using previously validated protocols. The WGA products from MDA were subjected to fluorescent PCR analysis to check the accuracy of WGA by MDA at 6 microsatellites. MDA products coming from single fibroblasts (which had previously been amplified) were also used to evaluate the PCR technique. Genomic DNA samples were used as positive controls. The MDA products were also used for CGH and microarray analysis to check whether known aneuploidies could be detected. The results of MDA products in CGH and microarrays were also compared with DOP products.

Results: (1) Microsatellite analysis: PCR analysis showed that MDA products from single buccal cells and clumps (groups of 5–10 cells) gave accurate allele sizing in only 17.6% of loci tested, compared with the accuracy of genomic DNA (98%) and MDA products from genomic DNA and single fibroblasts (100%). Allele dropout (ADO) was present at a rate of 15.7% in MDA products from buccal cells, 7% in single buccal cells or clumps that were directly subjected to PCR. ADO was absent in genomic DNA and MDA products derived from single fibroblasts. (2) CGH: Analysis was possible from all the MDA products derived from genomic DNA (6/6) however the results were less clear than when compared with DOP-PCR as a method for WGA. When single buccal cells were used, MDA did not provide any results after CGH (0/4). (3) Microarrays: When 200 ng of genomic DNA was applied to microarrays it presented good results. However, the same amount of MDA and DOP products gave unreliable results with high background noise (0/5).

Conclusions: According to a growing body of literature, MDA seems more advantageous compared with other techniques of WGA when using 10 ng of DNA as a starting template. However, when the starting template is from a single cell results are not as unequivocally positive. Based on the results in the present study, we speculate that the cell type from which the single cell DNA template is derived may play a crucial role in the accuracy, reliability and reproducibility of the results. Specifically, buccal cells may be suboptimal in comparison to fibroblasts for the purposes of WGA using MDA. Further optimization and laboratory-specific validation may be required before this technique can be applied routinely in clinical PGD.

O-109 Strategies and outcomes of preimplantation genetic diagnosis of familial adenomatous polyposis

S. Viville[1], N. Gardes[2], C. Moutou[2]

[1]*Institut de Génétique et de Biologie Moléculaire et Cellulaire, CNRS/INSERM/ULP, Illkirch, CU de Strasbourg., France;* [2]*SIHCUS-CMCO CHU de Strasbourg, Service de Biologie de la Reproduction, Schiltigheim, France*

Introduction: Preimplantation genetic diagnosis (PGD) was initially proposed to couples at risk of having a child affected by a severe genetic disorder (such as cystic fibrosis, myotonic dystrophy, spinal muscular atrophy). Fifteen years after the first PGD birth, new classes of indications emerged. These concern late onset diseases like Huntington's disease and, more recently, inherited cancer. Because of the adulthood onset of hereditary cancer, prenatal diagnosis (PND) raises numerous issues on the acceptability to terminate an affected pregnancy. This is the reason why PND for these disorders is generally considered as unacceptable by couples as well as geneticists and legal or ethical authorities. PGD for hereditary cancer, even if subject to controversy, seems to be a more acceptable option. We present our experience of PGD for familial adenomatous polyposis (FAP) between 2000 and 2005.

Materials and methods: Twelve couples were referred between 2000 and 2005. Average female age at intake was 29.4 years (vs. 31.4 for all referrals). Nine different mutations were involved, the recurrent 5 bp deletion at codon 1309 being present in 4 families. A family history was described in ten cases and two mutations occurred de novo in the affected partner. We initially developed PGD tests to detect the mutation alone, but we rapidly set up multiplex PCR combining mutation detection and indirect diagnosis using microsatellites D5S2027, D5S1965, D5S346, D5S421. We set up duplex and triplex indirect diagnoses to propose a PGD whatever mutation is involved in family cases. Protocols were optimized by modifying standard conditions, particularly after a judicious primer choice, in order to add internal controls in single cell PCR. Mutation detection strategies were based on: (i) sizing PCR fragment for deletions; (ii) restriction length polymorphism, when the mutation introduces a new restriction site; (iii) a new double allele specific PCR approach allowing the simultaneous detection of the wild type and mutated allele. PCR conditions were optimized on a minimum of 60 single lymphoblasts from control cell lines or from single lymphocytes from affected patients.

Results: Seven different protocols were set up—two simplex, four duplex combining direct plus indirect diagnosis and one triplex indirect diagnosis. Once PCR conditions were optimized, 60 to 114 single cells were tested with an amplification rate ranging from 95 to 100%. A complete genotype was obtained in 94% of cells with a PCR signal (81 to 99%) and a conclusive result in 99% (95 to 100%). ADO occurred in 5% of heterozygous cells (1 to 14%). Ten cycles were started, 9 reached the stage of biopsy and 7 had an embryo transfer procedure. A total of 75 blastomeres from 39 embryos were analyzed and 65 (87%) blastomeres from 36 embryos gave a result. A total of 11 untransferred embryos were reanalyzed and all confirmed PGD results. A positive HCG was obtained for 4 cycles: two biochemical and two ongoing pregnancies. A boy was born but no prenatal or postnatal testing was performed to confirm PGD results. A single clinical pregnancy is still ongoing.

Conclusion: We are now able to propose PGD to most couples at risk of transmitting FAP to their offspring, whether the mutation is inherited or occurred de novo. Our current strategy is to use to multiplex PCR combining either mutation detection and indirect diagnosis using microsatellites or indirect diagnosis for inherited cases with a private mutation. With our growing experience in workup, development of new protocols is less and less time consuming which enable us to work on specific PGD tests for unavailable de novo mutation.

O-110 Elective single embryo transfer compared with elective double embryo transfer in preimplantation genetic diagnosis

P. Donoso[1], W. Verpoest[1], E.G. Papanikolaou[1], I. Liebaers[2], A. Van Steirteghem[1], P. Devroey[1]

[1]*University Hospital Dutch-speaking Brussels Free University, Centre for Reproductive Medicine, Brussels, Belgium;* [2]*University Hospital Dutch-speaking Brussels Free University, Centre for Medical Genetics, Brussels, Belgium*

Introduction: Multiple pregnancies represent the main complication of ART. Since the 1st of July 2003 the new Belgian legislation imposes on women less than 36 years a restriction to single embryo transfer (SET) in their first treatment cycle. Although this strategy has shown to be effective without affecting the pregnancy rates, concerns have been raised on its possible adverse effect on the outcome of preimplantation genetic diagnosis (PGD) for inherited diseases (monogenic disorders and translocations), since a lower pregnancy rate has been observed compared with conventional IVF cycles. The aim of this study is to asses the impact of this legislation on the outcome of PGD cycles for monogenic disorders and translocations.

Materials and methods: We conducted a retrospective analysis of ICSI cycles with PGD for monogenic disorders and translocations, performed at our institution between January 2002 and December 2004, in women less than 36 years of age in their first treatment. Two groups of patients were compared: The elective double embryo transfer group (e-DET group), performed between January 2002 and June 2003 (before the new legislation) vs. the elective single embryo transfer group (e-SET group), performed since the implementation of the new law (July 2003 to December 2004). The main outcome measures to be compared among the two groups where delivery rate and multiple pregnancy rates. A separate analysis was also conducted for both monogenic disorders and translocations. Statistical analysis was performed using the Fischer's exact test.

Results: Fifty cycles were included in the e-DET group and 55 cycles in the e-SET group. The mean±SD for age (30.2±2.7 vs. 29.7±3.1), number of

cumulus-oocyte complexes (16.84±10.1 vs. 16.15±9.1) and number of zygotes (13±7.6 vs. 12.5±7.2) were comparable between both groups. The two main indications in the monogenic disorder group were myotonic dystrophy (12% e-DET group vs. 12.8% e-SET group) and cystic fibrosis (14% e-DET group vs. 3.6% e-SET group). Translocations represented 20% (n=10) of the e-DET group and 30.9% (n=17) of the e-SET group. There was no statistically significant difference in the delivery rates between the e-DET and the e-SET (32% vs. 25.5% p=0.52). Multiple pregnancies were eliminated when e-SET was performed (0% vs. 31.3% p=0.45). When the cycles for translocations were excluded—only monogenic diseases analyzed—the delivery rates became almost identical (30% e-DET vs. 28.9% e-SET, p=1.0). On the other hand, when cycles for translocations were exclusively analysed a higher delivery rate was achieved with e-DET than e-SET (40% vs. 17.6% p=0.36), though it did not reach statistical significance.
Conclusions: The implementation of a SET policy on young women undergoing PGD for monogenic disorders enables a significant reduction of multiple pregnancies without affecting the delivery rate. However, when PGD is performed to select out translocations, the efficiency of single embryo transfer might be debatable and needs further evaluation in a higher series.

FREE COMMUNICATION

Session 30 – Endometrium and endometriosis

Tuesday 20 June 2006 **10:00–11:30**

O-111 Multislice spiral computerized tomography after colon distension with water enteroclysis in the diagnosis of bowel endometriosis

S. Ferrero[1], E. Biscaldi[2], E. Fulcheri[3], N. Ragni[1], V. Remorgida[1], G.A. Rollandi[2]
[1]San Martino Hospital and University of Genoa, Department of Obstetrics and Gynaecology, Genoa, Italy; [2]San Martino Hospital and University of Genoa, Department of Radiology, Genoa, Italy; [3]San Martino Hospital and University of Genoa, Di.C.M.I. Unit of Anatomy and Histopathology, Genoa, Italy

Introduction: Although several radiological techniques have been proposed for the diagnosis of bowel endometriosis, data are inconclusive and no golden standard is currently available. This prospective study investigates the efficacy of multislice spiral computerized tomography after colon distension with a water enteroclysis (MSCTe) in the diagnosis of bowel endometriosis.
Materials and methods: Ninety-eight women with symptoms suggestive of colorectal endometriosis underwent MSCTe. To reduce bowel peristalsis and colonic spasm, 20 mg of joscine N-bromuro (Buscopan; Boehringer Ingelheim, Florence, Italy) were administered intravenously immediately before the water enema. Colonic distension was achieved by introducing 2000–2300 ml of water (37°C). All patients received an intravenous injection of Iopamidol (Bracco, Milan, Italy) with an iodine concentration of 370 mg/ml. The rate of intravenous injection of contrast material was set at 2.5 ml/s with an automatic power injector for all examinations. Bolus-tracking software designed to monitor organ contrast enhancement (SmartPrep; GE Medical Systems, Wisconsin, USA) was used to maximize the quality of MSCTe image. All patients were scanned on a 16-row MSCT scanner (LightSpeed, GE Medical Systems, Wisconsin, USA). The scan parameters were 16×0.625 mm collimation; rotation time 0.7 s; tube voltage 120 kV; effective mAs 370. The axial plane and multiplanar reconstructions were evaluated; images were independently reviewed at a PACS (picture archiving and communications system) workstation by two observers; disagreement between observers was resolved by consensus in a joined session. Locations, number of nodule/s, size of the nodule/s, and depth of bowel wall infiltration were determined. Within 20 days after the radiological examination, independently from the findings of MSCTe, all women underwent laparoscopy. At surgery, after adequate adesiolysis, terminal ileum, cecum, sigmoid colon and rectum were systematically examined to verify the presence of endometriotic lesions; all visible bowel endometriotic lesions were removed. MSCTe findings were compared with surgical and histological results.
Results: The endometriotic nature of all the bowel lesions removed at surgery was confirmed at histology. Laparoscopy confirmed the absence of bowel endometriosis 22 out of 23 women with entirely normal colon at MSCTe. In one patient with complete obliteration of the pouch of Douglas caused by a recto-vaginal nodule, MSCTe did not identify rectal involvement infiltrating the muscular layer (1.4 cm). Among subjects with a diagnosis of bowel endometriosis at MSCTe (n=75), additional four nodules were identified at surgery; they were all located on the rectum and reached the serosa in two cases and the muscolaris in other two cases. Therefore MSCTe identified 94.8% (110/116) of the bowel endometriotic nodules observed at surgery. MSCTe identified all nodules located on sigmoid colon, cecum and ileum; 47 out of 52 (90.4%) rectal nodules were diagnosed. MSCTe correctly determined the degree of infiltration of the bowel wall in all the identified serosal bowel nodules. In 6 nodules reaching the submucosa, the depth of infiltration was underestimated by MSCTe. A statistically significant positive correlation was observed between the diameter of the endometriotic nodules estimated at MSCTe and that measured by the pathologist (Pearson's Correlation Coefficient, r=0.974; p<0.001). MSCTe had a sensibility of 98.7%, a specificity of 100%, a positive predictive value of 100%, and a negative predictive value of 95.7% in identifying women with bowel endometriosis.
Conclusions: MSCTe is effective in determining the presence, size and depth of bowel endometriotic lesions.

O-112 Peritoneal macrophage depletion by liposomal bisphosphonate in a rat model of endometriosis affects both cytokines and macrophage infiltration

M. Schachter[1], H.D. Danenberg[2], S. Friedler[1], A. Raziel[1], S. Mendelovic[3], R. Ron-El[1], D. Strassburger[1], O. Bern[1], G. Golomb[4]
[1]IVF and Infertility Unit, Assaf Harofeh Medical Center Tel Aviv University, Zerifin, Israel; [2]Hebrew University Hadassah Hospital, Cardiovascular Research Unit, Jerusalem, Israel; [3]Dept. Pathology and Cytology, Assaf Harofeh Medical Center Tel Aviv University, Zerifin, Israel; [4]Hebrew University Jerusalem, Faculty of Pharmacology, Jerusalem, Israel

Background: Activation of macrophages and peritoneal inflammation is central to the initiation, implantation and perpetuation of endometriosis. Depletion of macrophages and monocytes can be achieved by liposome mediated intracellular delivery of biphosphonates (LBP) such as alendronate, which inactivate phagocytotic cells. We previously examined the effect of LBP on development of endometriosis in a rat model and found a significant reduction of implant volume and adhesion formation after treatment with LBP. Our objective in this study was to examine the effect of LBP on peritoneal cytokines and on endometriosis implant histology.
Material and methods: Twenty-four adult female Sabra strain rats were subjected to an endometriosis model, by resection of one uterine horn, and suture of 6 open uterine squares to the mesentery in each rat. Three additional rats were subjected to a sham operation which included resection of the uterus and nylon suturing to the mesentery with no uterine squares. The 24 rats were then divided randomly to two treatment and one control group, and treated with 4 weekly intraperitoneal injections of alendronate in liposomes. Two treatment doses were employed, 1 mg/kg per injection (low dose) and 10 mg/kg per injection (high dose). Four weeks after the initial surgery, the rats were sacrificed. Histopathological sections and avidin-biotin immunohistochemistry with staining for macrophages in implants were carried out with mouse anti-rat macrophage antigen ED1. The density of macrophage infiltration was scored by counting stained macrophages per HPF, expressed as the average number of stained macrophages counted per 800 background cells. Cell-free peritoneal fluid was collected at the time of sacrifice, and subsequently analysed for concentrations of tumor necrosis factor alpha (TNFα) and monocyte chemotactic protein 1 (MCP1) using commercially available ELISA kits.
Results: Immunohistochemistry demonstrated a significantly reduced pattern of macrophage infiltration in the low-dose treatment as opposed to the control group, (staining score 107±144 vs. 300±124, p<0.02) although no significant differences were found between the high-dose and control group for infiltration score (320±174 vs. 300±124). Peritoneal TNFα was significantly reduced in the high-dose group as opposed to low-dose and control groups, 0.52±1.03, 8.27±12.04 and 7.97±8.6 pg/ml, for high dose, low dose and control groups,