is now a preferred alternative for our centre with respect to PGD for chromosome rearrangements as it reduces the waiting time for treatment, negates the technical limitations of FISH technology which can confound diagnosis, and also allows the diagnosis of additional aneuploidies.

O15 Extended culture is a poor tool for selecting chromosomally normal embryos: Comparison of day-3 and day-5 embryos analyzed using comprehensive chromosome screening methods

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Aim: Most previous studies of blastocyst chromosome abnormalities were performed on embryos cultured using the prior generation of media, rather than the more complex formulations typically used today. In addition, cells were analyzed by FISH, which did not permit all of the chromosomes to be evaluated. The aim of this study was to compare comprehensive chromosome analysis, either by CGH or array-CGH, of day-3 and blastocyst stage embryos, to determine if extended culture can provide a useful screening tool for selecting euploid embryos.
Methods: 597 embryos underwent single cell biopsy on day-3 and were analyzed by array-CGH. A further 892 embryos had blastocyst biopsy on day-5 and analysis using CGH. The results were stratified by maternal age and compared.
Results: The results are summarized in the table. Monosomies, trisomies and complex abnormalities were all compatible with development to the blastocyst stage. For patients over 34 years of age the percentage of embryos affected by aneuploidy was significantly lower on day-5 compared with day-3 ($p < 0.0001$), but 58% of embryos were still abnormal.
Discussion: Culture to the blastocyst stage was associated with a small, but significant, increase in the proportion of euploid embryos. There was little if any affect effect in young patients, but a more substantial increase for women 35 and older. Yet even after extended culture, 47–60% of embryos from women over 35 were chromosomally abnormal. In conclusion, culture to the blastocyst stage provides a weak selection in favor of chromosomally normal embryos, but the majority of aneuploid embryos are capable of forming morphologically normal blastocysts.

	29–34		35–39		40-&-older	
	day 3	day 5	day 3	day 5	day 3	day 5
Normal	98 (46%)	82 (43%)	52 (33%)	187 (53%)	50 (22%)	110 (31%)
Abnormal	113 (54%)	107 (57%)	106 (67%)	165 (47%)	178 (78%)	241 (69%)
Total	211	189	158	352	228	351

O16 Comparative genomic hybridisation (aCGH) to identify unbalanced products associated with specific chromosomal rearrangements

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Aim: We describe use of array comparative genomic hybridisation (aCGH) to identify unbalanced products associated with specific chromosomal rearrangements.
Method: Embryos, single and multiple blastomere biopsies were analysed for confirmation of diagnosis or clinical PGD for two couples in whom the male partner carried a chromosomal rearrangement: (1) 46,XY,t(2;22)(p12;q11.2) and (2) 46,XY,t(3;11(q23;q24). Samples collected in 1–2 μl PBS on ice were subjected to whole genome amplification (WGA). Amplified test and control DNAs were reciprocally labelled with Cy3 or Cy5 before hybridization together on optimised BAC microarrays – CytoChip v3.1 and analysis using BlueFuse software (BlueGnome Ltd, Cambridge UK).
Results: WGA and aCGH was successful in 95% (36/38) samples. Couple 1: In cycle 1, of 8 embryos diagnosed as unbalanced by FISH, 5 were concordant with respect to the chromosomes involved in the rearrangement while 3 were euploid (FISH false positives). In cycle 2, multiple samples taken from 2 embryos diagnosed as unbalanced by FISH yielded array results with complete concordance between samples and with the FISH result. In cycle 3, following single blastomere aCGH, 9/13 embryos were diagnosed as unbalanced, 6 of which also showed multiple aneuploidies for unrelated chromosomes, 3 embryos were balanced but with multiple aneuploidies for unrelated chromosomes and 1 embryo yielded no result. Couple 2: 3 embryos diagnosed as unbalanced by FISH were confirmed as unbalanced by aCGH. The remaining 4 embryos (no FISH diagnosis) were apparently balanced but revealed multiple unrelated aneuploidies by aCGH. For both rearrangements, the translocation breakpoints were identified blind (i.e. directly from the embryo samples without prior knowledge of the parental karyotype).
Discussion: Array CGH accurately identifies not only unbalanced products associated with the rearrangement for which the test is indicated but also aneuploidies responsible for implantation failure, viable trisomies and miscarriage. Furthermore, no patient-specific test development is required and the test turnaround time allows day 5 embryo transfer.

O17 Whole genome profiling of early blocked embryos using customised genomic micro array

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Aim: Genomic and/or expression micro array technology became a common tool for genome and gene investigation in research & development and clinical diagnostic. For genome profiling and high resolution molecular karyotyping, array comparative genome hybridisation (array CGH) methods appear to be far better than classical CGH as they do not suffer from dependence on having metaphase preparations and have much higher sensitivity and specificity for subtle genomic changes. This study was focused on micro array application after whole genome amplification to assess the entire genome analysis of early blocked embryos regenerated from ART programme.
Material and methods: 42 patients undergoing ICSI programme were included in this study. Genomic DNA was extracted from 57 zona pellucida free embryos blocked at day 2–3. The DNA samples were submitted to whole genome amplification (WGA). Reference DNA samples prepared from few somatic cells following the same steps. Embryos and somatic cells were collected in 0.5 ml PCR tubes containing 5 μl of lysis buffer and incubated at 45ºC for 15 min, followed by proteinase K inactivation at 96ºC for 20 min. Lysates were used directly for whole genome amplification using Phi 29 kit, by adding 45 μl of the master mix in a total volume of 50 μl. The mix was then incubated at 30ºC for 6 hours followed by heat inactivation at 65ºC for 3 min. WGA products were labelled by random priming (test-Cy3 and control-Cy5, and the converse), as well as Cot-1 blocking DNA, are mixed and precipitated together. The labelled probes DNA solutions were hybridised to a BACS customised

constitutional genomic micro arrays (Eureka project E3802) for 16 hrs. A post hybridisation wash of the arrays was performed and then the arrays were scanned on a two-colour fluorescent scanner and the images were analysed using specific software.
Results and discussion: The data revealed that the use of WGA to amplify a minimum of 2–6 blastomeres gives rise to high quality and quantity genomic DNA, without preferential amplification. The micro array analysis provides suitable data for 46 embryos (46/57). As for the remaining embryos we couldn't conclude because of improper hybridisation quality and noisily profile, probably due to WGA efficiency. 19 embryos out of 46 (nearly 41%) turned out to bear chromosomes abnormalities. Unlike FISH, micro array allows the detection of simple and complexe aneuploidies, and also unbalanced and cryptics disorders. Still, these observations need to be confirmed by other molecular techniques. In practice Array CGH has its own limits, which include the inability to detect polyploidy or balanced chromosome abnormalities.
Conclusions: If we resolved the major problems of WGA in single or double blastomere: preferential amplification and Allele Drop Out. Genomic micro array CGH could be a new alternative to the aneuploidy testing by FISH and multiple genes analysis in Preimplantation Genetic Diagnosis.
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O18 Comparison of differential gene expression profile between human embryo on day 3 and trophoblast of blastocyst on day 5: Molecular signature specific for each

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Aim: The aim of this study was to compare the transciptome of the human TE with that of the embryo (day-3) in order to identify the genes involved in the TE specification and in initiation of pluripotency.
Methods: Five human TE samples were separated from the ICM under dissecting microscope and two donated embryos (day-3) were collected after informed consent from patients participating in *IVF* programmes. The present research programme was approved by the French Agence de la biomédecine. Each sample was individually analyzed by Affymetrix Genechip human micro-arrays (HG-U133 Plus 2.0). Statistical analysis was carried out with Significance Analysis of Microarrays (SAM) method with 2-fold cut-off and FDR <5%.
Results: Out of 25,970 analysed transcripts a list of 910 genes were significantly over-expressed in TE samples. This molecular signature comprised TE-specific genes such as *GATA3* ($\times$704, $p < 0.02$), cytoskeleton protein keratin 18 ($\times$524.2, $p < 0.02$), CD genes including *CD53* ($\times$50.7, $p < 0.02$), and new candidates that can serve as markers for human TE and many contribute to the TE development. The very high expression level of extracellular matrix related *LAMA1* ($\times$324.3, $p < 0.02$) in TE suggested that this laminin alpha1 could play an important role in TE specification. Conversely, human embryo day-3, in addition to known genes such as *DPPA2* and *MBD3L2*, over-expressed numerous different zinc finger transcription factors, including *ZNF595*.
Conclusion: The present study describes the first genome-wide overview of the specific feature of human TE and embryo transcriptional profiles, and provides fundamental resources for understanding the molecular network controlling human trophoblast specification and pluripotency. In addition, our results establish a solid basis for future studies in early embryo development that will help to identify candidate bio-markers for blastocyst viability. The understanding of this process opens a way to applications in *IVF*.

O19 Gene expression profile of apoptosis regulators of the Bcl-2 family during early embryonic development

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Aim: In women, more than 90% of the oocytes generated during fetal life are destined to die by apoptosis. Apoptosis have also been reported during early embryo development and is involved in cases of fragmentation and/or early developmental arrest. The Bcl-2 family comprises both anti and proapoptotic proteins that regulate cell death/survival and are likely to play a determinant role in controlling oocyte and early embryo demise. The aim of the present study is to evaluate by DNA microarray the expression of Bcl-2 family genes in human oocytes, embryos on day 3 (d3) and at blastocyst stage.
Method: The transcription profile of human mature MII oocytes (n = 9), d3 embryos (n = 2), trophectoderm cells (TC) (n = 5) and embryonic stem cells (hESC) (n = 8) was determined using Affymetrix Human Genome U133 Plus 2.0 GeneChip arrays (Santa Clara, CA). This study has received institutional approval. Informed consent was obtained from all subjects. A consensus-list of Bcl-2 family genes was obtained by review of Medline and comprises 21 genes. Based on our microarray data we investigated the expression of selected genes during early embryonic development. Statistical comparisons were made with the non-parametric Kruskal–Wallis test. p-values
Results: The prosurvival factor Bcl2l10 ($\times$27.4; p = 0.0003) and its proapoptotic homolog Bcl2l13 ($\times$6;p = 0.0003) are highly expressed in the oocyte. We observed that the antiapoptotic member Mcl-1 ($\times$12; p = 0.0004) and the proapoptotic Bh3-only members Bcl2l11 ($\times$18; p = 0.0091) and Bik ($\times$16; p = 0.0145) are induced on d3 embryos. In TC as well as in hESC, Mcl-1 ($\times$15 and $\times$19 respectively; p = 0.0004) remains over-expressed. Several proapoptotic members are detected but at lower levels. The expression of these members will be discussed in relation to oocyte quality and embryo developmental competence.
Conclusion: Most of the existing studies analyzed a limited number of members with a focus on the expression of antiapoptotic Bcl-2 and proapototic Bax. The concomitant analyses of all family members allowed by the microarray approach revealed that other homologs are differentially expressed in oocytes and early embryos under in vitro culture conditions.