Hum Genet (1997) 101 : 30–36

ORIGINAL INVESTIGATION

Hazel J. Clouston · Jeanette Fenwick · Alison L. Webb
Mary Herbert · Alison Murdoch · John Wolstenholme

Detection of mosaic and non-mosaic chromosome abnormalities in 6- to 8-day-old human blastocysts

Received: 22 April 1997 / Accepted: 27 May 1997

Abstract A reliable technique has been developed for the production of good quality G-banded chromosome preparations from 6- to 8-day-old human blastocysts (20–800 cell stage) from an in vitro fertilization programme. The technique involves a thymidine cell division synchronization step to reduce the exposure time to colcemid, in conjunction with a simple 70% acetic acid disaggregation procedure to produce discrete metaphases for analysis. Of 105 blastocysts processed by this technique, 9 were lost during handling and 10 showed no dividing cells. The remaining 86 produced useful separate metaphases with a mean mitotic activity of 6.5%. A full G-banded karyotype was obtained from 1–6 cells in 55 blastocysts (64%), incomplete G-banded analysis but with full information of ploidy was obtained from 18 blastocysts (21%), with 13 (15%) producing no useful cytogenetic results. Abnormalities observed included polyploidy, diploid/polyploid mosaicism, non-mosaic trisomy 16 (2 cases), 46,Xdel(X)-(q21)/46,XX (1 case) and several single cells with trisomies or structural anomalies in otherwise normal blastocysts. Variable levels of structural chromosome damage, with apparent interchanges, chromosome branching and anomalous chromatid pairing were also seen.

Introduction

The delineation of the extent and nature of mosaic and non-mosaic chromosome abnormalities in early human preimplantation embryos is important for understanding the origins and selective processes leading to the anomalies seen later in gestation. This includes true fetal anomaly and confined placental mosaicism (CPM), in both continuing and non-continuing pregnancies. Although some limited but useful karyotypic information can be obtained by processing intact 2- to 3-day-old embryos (Angell et al. 1986; Plachot et al. 1987; Papadopoulos et al. 1989; Jamieson et al. 1994), the quality of metaphases and G-banding obtained is at best sub-optimal and certainly well below that which can be obtained in routine diagnostic preparations from blood, amniotic fluid and tissue fibroblast cultures.

In addition to the basic technical difficulties of physically handling individual preimplantation embryos, several competing factors essentially compromise the potential quality of preparations and hence limit the degree of information that it is realistic to expect (Zenzes and Casper 1992). At the 1- to 8-cell stage, long colcemid exposure times can be used to raise the mitotic index (MI), but this produces highly contracted chromosomes that often exhibit chromatid separation and that G-band poorly, if at all. At these earliest stages, however, there is at least the possibility that some discrete metaphases will be seen. At later developmental stages, viz. 20–100 cells, where there is the potential of a greater yield of metaphases with some realistic chance of being able to detect mosaicism, the now compact nature of the preimplantation embryo means that dividing and non-dividing cells are often superimposed, obscuring enough metaphases so that any benefit is lost. In addition, metaphases are often either poorly spread or over-spread and dispersed, depending on their location at the centre or the periphery of the resulting preparations, respectively (Herbert et al. 1995). Furthermore, the poorly spread cells are often not in a single focal plane, again restricting the potential for analysis. Interphase fluorescence in situ hybridization (FISH), an alternative approach avoiding the need for metaphase preparations, has been applied successfully to 2- to 12-cell stage embryos, both as intact entities (Harper et al. 1995) and biopsied blastomeres (Munné et al. 1995). However, the current technical limitations of multi-colour FISH restrict the number of chromosome homologues that can be efficiently assessed simultaneously (Delhanty and Handyside 1995).

H. J. Clouston · A. L. Webb · J. Wolstenholme (✉)
Department of Human Genetics,
University of Newcastle upon Tyne, 20 Claremont Place,
Newcastle upon Tyne, NE2 4AA, UK
Fax: +44-191-222-7194

J. Fenwick · M. Herbert · A. Murdoch
Centre for Reproductive Medicine, Royal Victoria Infirmary,
Newcastle upon Tyne, NE1 4LP, UK

We describe a technique for producing small numbers of good quality, G-banded metaphases from 6- to 8-day-old human blastocysts, allowing the detection of both mosaic and non-mosaic abnormal karyotypes at this stage of human development. The technique is also compatible with FISH-based analyses of both metaphase and interphase cells. A preliminary analysis of the abnormalities observed is presented.

Materials and methods

Source

The embryos used were donated by couples undergoing in vitro fertilization (IVF) treatment in accordance with Human Fertilization and Embryology Authority regulations. The study was approved by the Joint Ethics Committee of Newcastle and North Tyneside Health Authority and the University of Newcastle upon Tyne.

Superovulation

Superovulation was achieved by a standard regime of gonadotrophin releasing hormone analogue (Suprefact; Hoechst, Hounslow, UK) and either human menopausal gonadotrophin (Pergonal; Serono UK, Welwyn Garden City, UK) or follicle-stimulating hormone (Metrodin; Serono UK) at a dose of 150–300 IU per day for 11–14 days, followed by 5000 or 10 000 IU human chorionic gonadotrophin (hCG, Profasi; Serono UK), 38–40 h prior to follicle aspiration. Oocytes were retrieved by ultrasound-guided aspiration of follicles at 38–40 h after hCG treatment.

Insemination and culture

Oocytes were cultured and inseminated in 100 ml droplets of medium under mineral oil (Sigma). The medium was Earle's balanced salt solution (EBSS; Life Technologies, Paisley, Scotland) supplemented with 25 mM sodium bicarbonate (Analar grade, BDH, UK), 0.47 mM sodium pyruvate (Analar grade, BDH) and 10% v/v of a 5% solution of human serum albumin (HSA, Albuminar-5; Armour Pharmaceuticals, UK). They were incubated in 5% CO_2 in air, at 37°C and with 99% relative humidity. Motile sperm were separated from semen by centrifugation on a discontinuous Percoll (Pharmacia, Sweden) gradient composed of 1.5 ml each of 90% and 45% Percoll solutions in HEPES-buffered EBSS solutions. Oocytes were inseminated 41–43 h after hCG treatment (day 0). On day 1, the cumulus cells surrounding the oocyte were mechanically removed either with finely drawn pipettes or needles. Only those with apparent normal fertilization, indicated by the presence of two pronuclei at this stage, were included in the study. These embryos were transferred to a fresh droplet of modified EBSS supplemented with 15% (v/v) HSA (5% solution) under mineral oil.

After 24–48 h of culture, embryos were morphologically assessed by using an inverted microscope at 200x magnification. The development of cleavage stage embryos was assessed by counting the number of blastomeres. The degree of cytoplasmic fragmentation and evenness of blastomere size was also noted. A maximum of three of the fastest growing, least fragmented embryos were selected for uterine transfer. The remaining embryos were transferred to 700 ml DMEM:Ham's F12 (1:1; Life Technologies), supplemented with 2% (v/v) Ultroser (Life Technologies), in 4-well dishes (Nuclon) and cultured for a further 4–6 days.

Embryos were classified as blastocysts when a blastocoelic cavity surrounded by a distinct layer of trophoblast cells was observed. Blastocyst expansion was subjectively assessed on the basis of volume increase and the degree of zona thinning.

Blastocyst harvesting and slide preparation

Six to eight days post-fertilization, 20% of normally fertilized embryos had developed into blastocysts. Of the 105 embryos reaching this point, only one remained unexpanded; 40% of blastocysts had also begun or completed the process of hatching from the zona pellucida (15% hatching, 25% fully hatched). Cell division was synchronized by incubation in medium supplemented with 0.5 μg/ml thymidine for approximately 16 h. They were then transferred to fresh medium and incubated for 2 h, before the addition of colcemid (final concentration 0.1 μg/ml) and culturing for a further 6 h. The blastocysts were placed in 0.3 ml hypotonic solution for 10 min. For hatched blastocysts, this was 1% sodium citrate; for expanded and unexpanded blastocysts, 1% sodium citrate:Bactotrypsin solution (Difco):water (18:1:1) was used. Bactotrypsin weakens the zona pellucida, aiding subsequent dissociation of the encapsulated cells. The blastocysts were slowly fixed by gradually adding 0.3 ml 3:1 methanol:acetic acid fixative. They were left at room temperature for 5 min and then transferred to 0.6 ml 3:1 fixative for at least 20 min. Individual embryos were then placed in 0.6 ml methanol:acetic acid:water (3:3:1) for 1–2 min to soften the cells and cause them to swell, before final disaggregation in 70% acetic acid. This was achieved by using a 100-μl micropipettor set at 25 μl. The blastocyst was taken up into the plastic tip in the smallest possible volume of 3:3:1 fix (approximately 3–5 μl) and then 70% acetic acid was drawn up behind to make a total of 25 μl. After 20 s, this solution was transferred to a polylysine-coated slide and observed under a dissecting microscope. If the blastocyst was not fully disaggregated, the acetic acid was gently pipetted up and down over it until dissociation of the blastomeres was complete. The slides were air-dried and either aged overnight at 60°C before G-banding analysis or aged overnight at room temperature prior to FISH analysis.

Analysis

For G-banding analysis, slides were first treated for 5 min with 2× Hanks' balanced salt solution. After being rinsed in saline (0.15 M NaCl), each slide was gently agitated for 15–20 s in trypsin solution, viz. 2 ml trypsin stock solution (Trypsin 1:250 Difco laboratories, 0.1 g/ml in water) in 20 ml pH 6.8 buffer (BDH) and 20 ml saline, before being rinsed twice more in saline. Slides were stained in 4% Giemsa stain/24% Leishman's stain in pH 6.8 buffer for 3 min, rinsed in water and blotted dry. Mitotic cells and interphase nuclei were scored for each blastocyst and the MI was calculated. FISH was performed according to the manufacturer's protocol with Oncor probes for X-cen (α-satellite probe DXZ1), X-cen and Yq12 (XY cocktail of DXZ1 and Y classical satellite probe DYZ1) and the DiGeorge region 22q11.2 (D22S75 with control loci probe D22S39).

Results

To date a series of 105 blastocysts has been processed. Availability for processing was dependent on individual embryos reaching the blastocyst stage as part of a separate project within the IVF programme and therefore beyond the control of this study. The majority (95) were synchronized on day 7 post-fertilization, whereas 5 were synchronized on day 6, and 5 on day 8. The success rate is summarized in Table 1. Nine blastocysts were lost during fixation. Most of those lost were comparatively small and appeared to have commenced degeneration. Ten blastocysts produced no metaphase cells, suggesting that active division had ceased. The subsequent analysis of results is based on the 86 blastocysts (82%) that provided mitotic cells.

Table 1 Summary of success rate in achieving metaphases of sufficient quality for full analysis, incomplete analysis or assessment of ploidy only

Total in seriers	105	
Lost during processing	9	
No metaphase cells	10	
Total blastocysts with metaphase cells	86	
Full G-banded karyotype available from 2–6 cells	27/86	(31%)
Full G-banded karyotype available from 1 cell only	28/86	(33%)
Partial analysis only, full information regarding ploidy	18/86	(21%)
Metaphases present but no analysis possible	13/86	(15%)

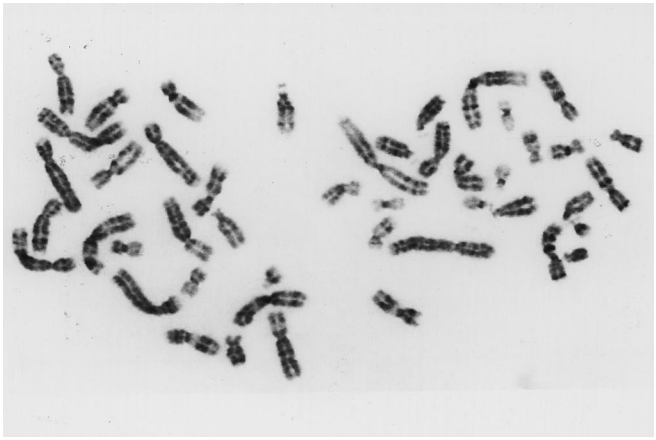

Fig. 1 Good quality metaphase from a 7-day-old expanded blastocyst

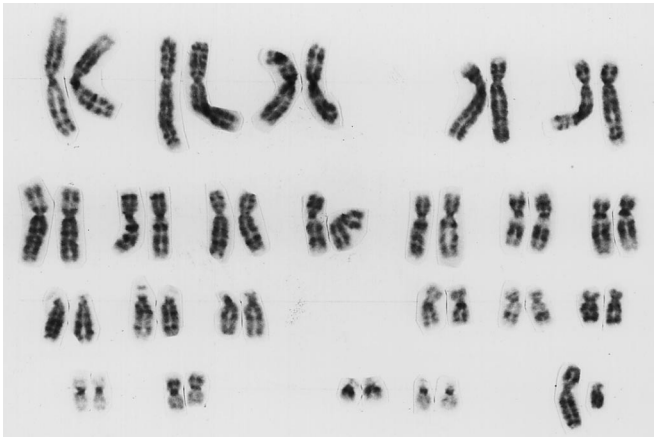

Fig. 2 Karyogram of the metaphase in Fig. 1

Total cell counts ranged from 20 to 819 (mean = 175, SD = 150), whereas MIs lay between 0.004 and 0.191 (mean = 0.065, SD = 0.044). The quality of the individual metaphases was somewhat variable but 64% of blastocysts generated at least one good quality, fully analysable, G-banded metaphase (Figs. 1, 2), with 31% producing between 2 and 6 full karyotypes; less informative dividing cells could be used for the confirmation or exclusion of previously identified abnormalities. In a further 21%, it

Table 2 Summary of incidence of polyploidy, non-mosaic trisomy and mosaicism

Analysis possible	73	
Polyploid (mainly tetraploid)	7/73	(10%)
Diploid/tetraploid mocaicism	17/73	(23%)
Diploid (including abnormals)	49/73	(67%)
Trisomy 16 (non-mosaic)	2/73	
Mocaicism (multiple cells):46,X,del(X)/46,XX	1/73	
Possible mosaicism (single trisomic cell plus normal cells)	3/73	

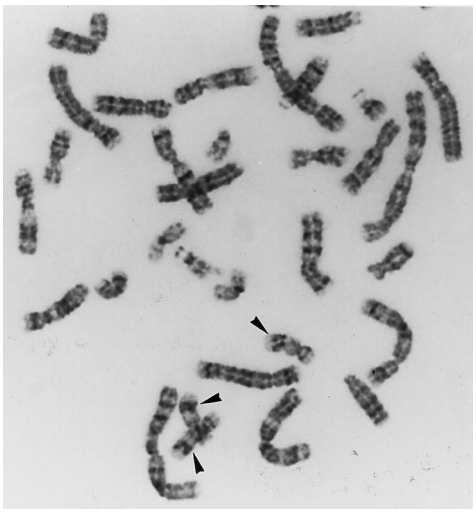

Fig. 3 A single cell exhibiting trisomy 17 (*arrowheads*) in an otherwise normal male blastocyst

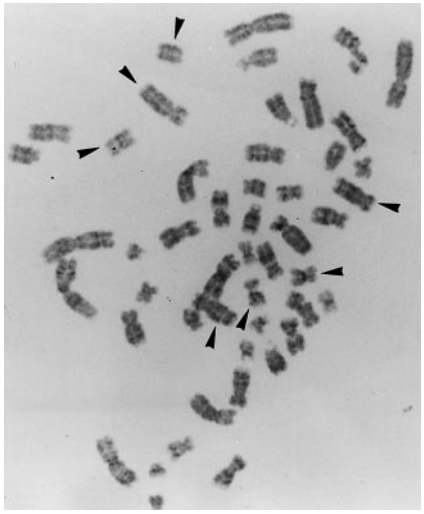

Fig. 4 A single cell from an otherwise trisomy 16 blastocyst, showing trisomy 5 and an acentric fragment as additional abnormalities (*arrowheads*)

was not possible to achieve a complete G-banding analysis of entire cells but the number of chromosomes could be scored and a partial analysis undertaken; full determination of ploidy could also be achieved. In 13/86 blastocysts, no detailed analysis was possible, because of over-

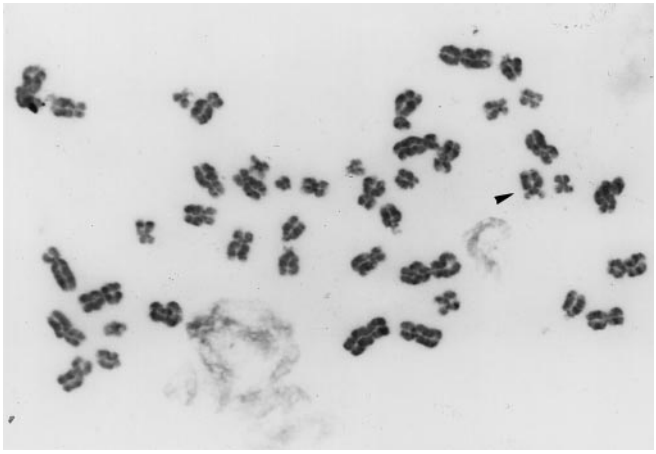

Fig. 5 A single cell showing t(14;22), which is indicated by an *arrowhead*. A further metaphase had an additional marker chromosome apparently derived from most of 14q, possibly as a result of a common chromosome damage event in an earlier cell cycle. The translocation was not present in two other cells from the same blastocyst

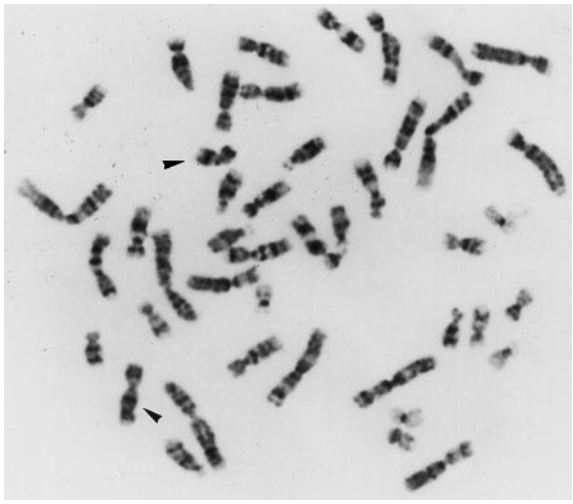

Fig. 6 Female with deletion of the long arm of one X chromosome (*arrowhead*); another cell from the same blastocyst showed the same anomaly, whereas three others had two normal X chromosomes

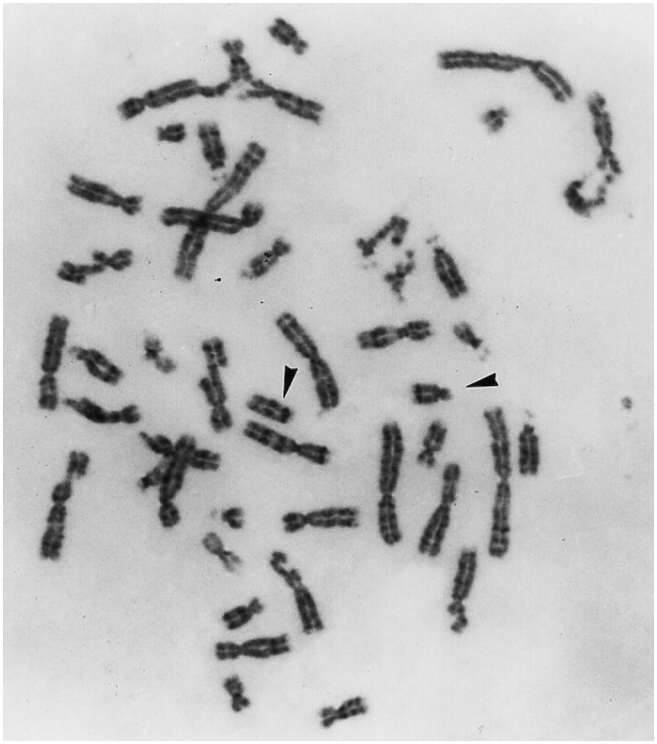

Fig. 7 A typical cell with chromosome breaks and interchanges. *Arrowheads* Deleted chromosome 7 and the resulting fragment

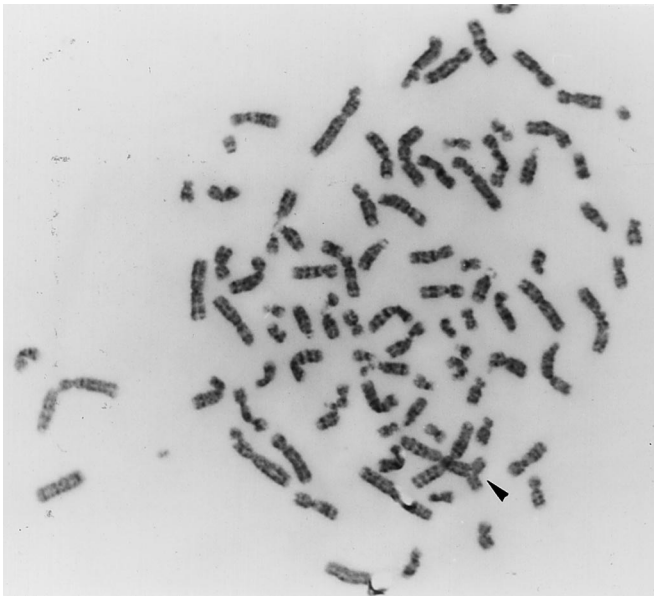

Fig. 8 A tetraploid cell with branching of distal 1p (*arrowhead*)

lapping chromosomes, contracted chromosomes with poor morphology or lack of intact metaphases (a problem related to artefactual damage during the acetic acid disaggregation stage). The results from the 73 blastocysts where analysis was possible are summarized in Table 2. Two thirds were diploid, 10% were tetraploid and 23% were diploid/polyploid mosaics. In the mosaic blastocysts, the polyploid cell line was usually tetraploid but occasionally much larger chromosome complements (e.g. 8n) were seen in single cells.

Of the diploid blastocysts, a proportion demonstrated chromosome anomalies of potential direct significance to relevant constitutional chromosome abnormalities in continuing pregnancies. Two cases of non-mosaic trisomy 16 were observed, with each of the three and four analysed cells, respectively, possessing an extra copy of chromosome 16. Three cases of single trisomic cells among apparently normal diploid cells were also detected (2 cases of a trisomy 17 cell and one case of a trisomy 4 cell; Fig. 3). One of the trisomy 16 blastocysts displayed one cell with an extra copy of chromosome 5 and an unidentified acentric fragment, in addition to the extra copy of chromosome 16 seen in the other two cells (Fig. 4). An appar-

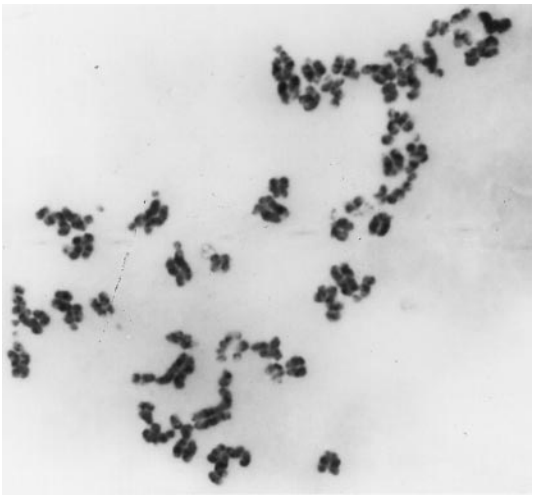

Fig. 9 Extreme chromosome damage in a small proportion of cells

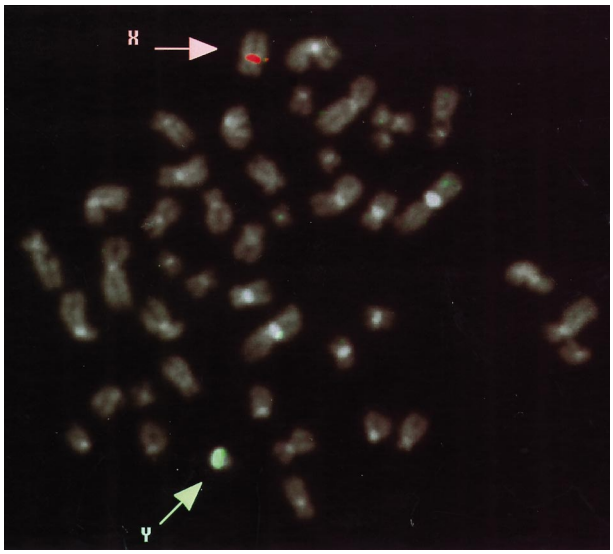

Fig. 10 Dual colour FISH of a male metaphase showing X-centromere-specific-satellite probe DXZ1 (*red*) and Yq12-specific satellite probe DYZ1 (*green*)

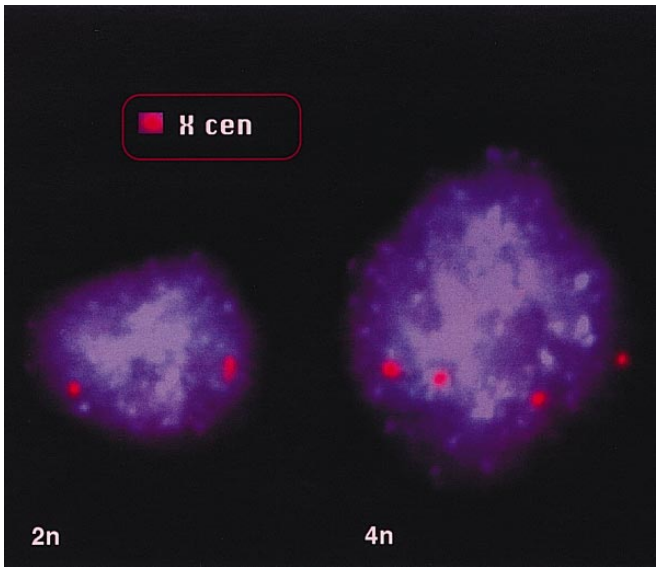

Fig. 11 Single colour FISH of interphase nuclei from a 2n/4n mosaic female blastocyst with probe DXZ1. The same mosaicism could be clearly seen in metaphase cells (not shown)

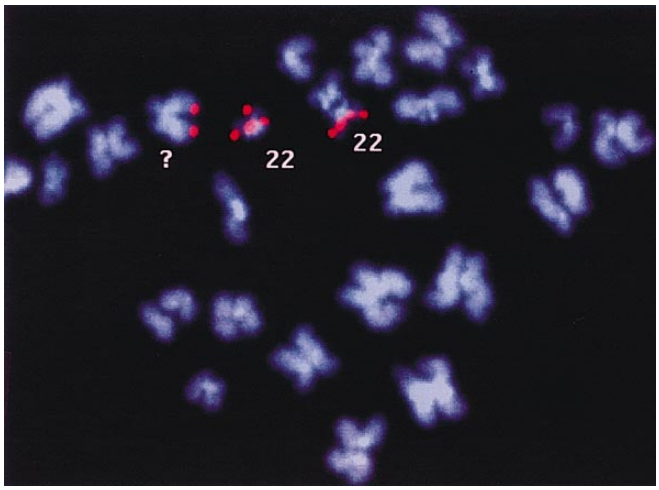

Fig. 12 FISH with a cosmid probe for the DiGeorge region of 22q11.2 (D22S75) and a control loci probe for distal 22q (D22S39). Two chromosomes 22, one in association with a chromosome 21, are visible. In this cell, one of the probes also detects a rearranged chromosome, not seen in other metaphases, but exhibiting partial duplication of 22q (*question mark*)

ent Robertsonian translocation t(14;22) was observed in one cell alongside others without the rearrangement (Fig. 5). Mosaicism for a partial deletion of the long arm of the X chromosome, 46,Xdel(X)(q21)[2],/46,XX[3], was clearly demonstrated in one preparation (Fig. 6).

Cells with other structural and numerical aberrations of unclear relationship to constitutional abnormalities were also observed. About 40% of blastocysts had cells with significant levels of unrepaired chromosome damage. This varied from single chromatid breaks and gaps to gross damage, with the majority of chromosomes being affected. Inappropriate repair, detected as interchanges between non-homologous chromosomes could be observed. Rearranged chromosomes, acentric fragments and branching of distal chromosome arm segments, suggestive of similar damage in a previous division cycle, was also present (Figs. 7–9). Hypodiploidy and hyperdiploidy were recorded. Some of this was undoubtedly artefactual, because of metaphase breakage during processing, but a number of karyotypes, e.g. 53,XX,+2,+5,+6,+10,+14,+18, +20,+20,-22, indicated true hyperdiploidy rather than the random fracture of a polyploid cell.

Although few blastocysts have currently been analysed by FISH, preliminary data suggest that this procedure can be successfully applied to cells generated from blastocysts by our technique. The sex of individual embryos has been determined by performing dual colour FISH with probes for X and Y chromosomes (Fig. 10). An X probe has been used to demonstrate diploid/tetraploid mosaicism in a fe-

male blastocyst, with 2 and 4 signals, respectively, being seen in both interphase (Fig. 11) and metaphase cells. Probe D22S75 mapping to the DiGeorge locus (22q11.2) and a control probe mapping to 22q13.3 demonstrate that single copy cosmid probes can also be applied to these preparations (Fig. 12). These blastocysts have not undergone conventional cytogenetic analysis and are not included in the series of 105 above.

Discussion

The number of blastocysts analysed so far is too small to allow any major conclusions regarding the absolute incidence of chromosome anomalies. The general pattern however is in agreement with observations in comparable series of 1- to 8-cell embryos and the incidence that can be deduced from the frequency seen later in pregnancy which must define a minimum anticipated presence at this early stage.

The observation of two cases of trisomy 16 in a series of 86 blastocysts is compatible with previous cytogenetic studies of 1- to 8-cell embryos giving incidences of 0/22 (Angell et al. 1986) and 3/178 (Jamieson et al. 1994) and a detection rate of 1 in 64 in FISH studies of biopsied blastomeres from embryos at a similar stage (Munné et al. 1995). It also fits with the observed incidence later in the first trimester, from which it can be estimated that approximately 1.5% of all recognized pregnancies start from conceptions trisomic for this chromosome, and with its well-established status as the most common trisomy, at conception, in man (see Wolstenholme 1995 for references).

Significant levels of tetraploidy, both mosaic with diploidy and non-mosaic, have been reported previously (Angell et al. 1987; Plachot et al. 1987; Jamieson et al. 1994; Munné et al. 1995; Harper et al. 1995) but the variation in the stage and quality of embryos studied and the diverse approaches used for analysis make direct comparison with our series difficult. Our ability to assess ploidy in larger numbers of cells will clearly increase the frequency at which low levels of tetraploid cells can be recognized. Mosaic tetraploidy may result from the production of bi-nucleate blastomeres by the failure of cytokenesis (Hardy et al. 1993) as part of an overall pattern of the relaxation of cell-division control processes. An association between mosaic tetraploidy and poor quality arrested embryos has been observed (Angell et al. 1987; Munné et al. 1995). Low numbers of tetraploid cells are also considered to be part of normal trophoblast development (Angell et al. 1987) giving rise to the tetraploidy seen at variable levels in cytotrophoblast cells, which are studied as part of prenatal diagnosis by means of chorion villus samples, where in general its presence seems benign (Association of Clinical Cytogeneticists Working Party 1994). Although triploidy may represent 1% of all conceptions, it has not been seen in this series. This may be a consequence of the small numbers of blastocysts analysed so far but may also reflect the exclusion of tri-pronuclear embryos as part of the IVF process.

Mosaicism has been recorded in cytogenetic studies of 2- to 3-day embryos (Papadopoulos et al. 1989; Jamieson et al. 1994) but the definitions used in these studies cover abnormalities such as gross hypodiploidy and hyperdiploidy, which are unlikely to be of significance to constitutional mosaicism. For this reason, the results are difficult to extrapolate to either mosaicism in the fetus proper or CPM in continuing pregnancies. Our case of apparent mosaicism for deletion of Xq, 46,Xdel(X)(q21)/46,XX, would, if present in a fetus, produce a variant form of Turner's syndrome. The more common mosaic trisomies, viz. for chromosomes 2, 3, 7, 8, 9 and 16, which are seen primarily in later continuing pregnancies as CPM, were not observed in this reported series of synchronized embryos, although an example of trisomy 2 (one cell)/normal male (one cell) was seen during the unreported developmental stage of the synchronization process. These mosaic abnormalities have individual observed frequencies of less than 1 in 1000 by the end of the first trimester (Wolstenholme 1996). This may be an underestimate of the overall incidence at the blastocyst stage, as it excludes the smaller numbers of cases recognized in early pregnancy losses (Hassold 1982; Warburton et al. 1978) and takes no account of unknown selection pressures for or against the proliferation of abnormal cells. The failure to detect any of these mosaic trisomies could however be anticipated within the limits of the numbers of synchronized embryos karyotyped so far.

The significance of our observations of single cells showing trisomy 4 and trisomy 17 is unclear. Other interphase cells may have the same anomaly. Similarly, if these cells are able to replicate, they would produce a subpopulation with the abnormality concerned. Either way, these cells could represent mosaicism relevant to constitutional karyotypes. Although both abnormalities are comparatively rare, trisomy 4 mosaicism has been recorded in spontaneous losses (Hassold 1982) and a liveborn (Marion et al. 1990); trisomy 17 mosaicism is known in spontaneous losses (Warburton et al. 1986), CPM (Kalousek et al. 1987; Teshima et al. 1992; Roland et al. 1994) and at term (Shaffer et al. 1996).

Papadopoulos et al. (1989) report almost identical levels of structural chromosome damage to that in this present series, with the same effects being described by both Angell et al. (1986) and Plachot et al. (1987). Suprisingly, these anomalies are not mentioned in Jamieson et al. (1994). Damage in early cell divisions may be a consequence of the ovarian stimulation stage of the IVF process rather than a characteristic of early preimplantation embryos per se (Elbling and Colot 1985) but it is unclear whether such effects would continue to produce new damage 7 days post-insemination after culture in vitro, as in this series. Chromatid and chromosome breaks and gaps are not considered to be a result of the thymidine synchronization stage of processing, as they have also been observed in this laboratory in a small parallel series of day-7 blastocysts processed following overnight exposure to colcemid without thymidine pretreatment (data not shown). The recorded levels of structural chromosome

damage would appear to be compatible with apparently normal in vitro development to the hatched blastocyst stage in our series. Useful analysis of the extent and nature of chromosome damage will require data from a larger group of embryos and this is in hand.

Hypodiploidy, hyperdiploidy and structural chromosome damage almost certainly reflect the same basic phenomenon, termed uncontrolled or chaotic division, predicted by FISH studies of interphase nuclei at earlier developmental stages (Harper et al. 1995; Munné et al. 1995). The potential for such cells to cause misinterpretation during preimplantation genetic diagnosis is well recognized (Delhanty and Handyside 1995). Without access to non-IVF blastocysts, the relevance to normal human development remains an open question.

To summarize, by using the technique described, it is now possible to investigate cytogenetic anomalies in spare IVF blastocysts. Alternative synchronization and cell disaggregation regimes are worthy of investigation but the general approach produces preparations suitable for both conventional cytogenetic and FISH analysis of mosaic and non-mosaic chromosome abnormalities at this stage of pregnancy.

Acknowledgements This work was supported by Newcastle University Hospitals Special Trustees (H.C.) and WellBeing Grant W3/94 (A.W.).

References

Angell RR, Templeton AA, Aitken RJ (1986) Chromosome studies in human in vitro fertilization. Hum. Genet 72:333–339

Angell RR, Sumner AT, West JD, Thatcher SS, Glasier AF, Baird DT (1987) Post-fertilization polyploidy in human preimplantation embryos fertilized in-vitro. Hum Reprod 2:721–727

Association of Clinical Cytogeneticists Working Party on Chorionic Villi in Prenatal Diagnosis (1994) Cytogenetic analysis of chorionic villi for prenatal diagnosis: an ACC collaborative study of UK data. Prenat Diagn 14:363–379

Delhanty JDA, Handyside AH (1995) The origin of genetic defects in the human and their detection in the preimplantation embryo. Hum Reprod Update 1:201–215

Elbling L, Colot M (1985) Abnormal development and transport and increased sister-chromatid exchange in preimplantation embryos following superovulation in mice. Mutat Res 147: 189–195

Hardy K, Winston RML, Handyside AH (1993) Binucleate cells in human preimplantation embryos in vitro: failure of cytokinesis during cleavage. J Reprod Fertil 98:549–558

Harper JC, Coonen E, Handyside AH, Winston RML, Hopman ANH, Delhanty JDA (1995) Mosaicism of autosomes and sex chromosomes in morphologically normal, monospermic preimplantation human embryos. Prenat Diagn 15:41–49

Hassold T (1982) Mosaic trisomies in human spontaneous abortions. Hum Genet 61:31–35

Herbert M, Wolstenholme J, Murdoch AP, Butler TJ (1995) Mitotic activity during preimplantation development of human embryos. J Reprod Fertil 103:209–214

Jamieson ME, Coutts JRT, Connor JM (1994) The chromosome constitution of human preimplantation embryos fertilized in vitro. Hum Reprod 9:709–715

Kalousek DK, Dill FJ, Pantzar T, McGillivray BC, Yong SL, Wilson RD (1987) Confined chorionic mosaicism in prenatal diagnosis. Hum Genet 77:163–167

Marion JP, Fernhoff PM, Korotkin J, Priest JH (1990) Pre- and postnatal diagnosis of trisomy 4 mosaicism. Am J Med Genet 37:362–365

Munné S, Alikani M, Tomkin G, Grifo J, Cohen J (1995) Embryo morphology, developmental rates, and maternal age are correlated with chromosome abnormalities. Fertil Steril 64:382–391

Papadopoulos G, Templeton AA, Fisk N, Randall J (1989) The frequency of chromosome anomalies in human preimplantation embryos after in-vitro fertilization. Hum Reprod 4:91–98

Plachot M, Grouchy J de, Junca A-M, Mandelbaum J, Turleau C, Coulin P, Cohen J, Salat-Baroux J (1987) From embryo to oocyte: a model, deduced from in vitro fertilization, for natural selection against chromosome abnormalities. Ann Génét (Paris) 30:22–32

Roland B, Lynch L, Berkowitz G, Zinberg R (1994) Confined placental mosaicism in CVS and pregnancy outcome. Prenat Diagn 14:589–593

Shaffer LG, McCaskill C, Hersh JH, Greenberg F, Lupski JR (1996) A clinical and molecular study of mosaicism for trisomy 17. Hum Genet 97:69–72

Teshima IE, Kalousek DK, Vekemans MJJ, Markovic V, Cox DM, Dallaire L, Gagne R, Lin JCC, Ray M, Sergovich FR, Uchida IA, Wang H, Tomkins DJ (1992) Chromosome mosaicism in CVS and amniocentesis samples. Prenat Diagn 12:443–466

Warburton D, Yu C-Y, Kline J, Stein Z (1978) Mosaic autosomal trisomy in cultures from spontaneous abortions. Am J Hum Genet 30:609–617

Warburton D, Kline J, Stein Z, Strombino B (1986) Cytogenetic abnormalities in spontaneous abortions of recognized conceptions. In: Porter IH, Hatcher WH, Willey AM (eds) Perinatal genetics: diagnosis and treatment. Birth Defects Institute Symposia, Academic Press, New York

Wolstenholme J (1995) An audit of trisomy 16 in man. Prenat Diagn 15:109–121

Wolstenholme J (1996) Confined placental mosaicism for trisomies 2, 3, 7, 8, 9, 16 and 22: their incidence, likely origins and mechanisms for cell lineage compartmentalisation. Prenat Diagn 16:511–524

Zenzes MT, Casper RF (1992) Cytogenetics of human oocytes, zygotes, and embryos after in vitro fertilization. Hum Genet 88: 367–375