Human Reproduction Vol.16, No.9 pp. 1954–1958, 2001

# Developmental ability of chromosomally abnormal human embryos to develop to the blastocyst stage

**M.Sandalinas[1], S.Sadowy, M.Alikani, G.Calderon, J.Cohen and S.Munné**

Gamete and Embryo Research Laboratory, The Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center, West Orange, NJ 07052, USA

[1]To whom correspondence should be addressed. E-mail: mireia.sandalinas@embryos.net

**BACKGROUND: A correlation between morphology, developmental competence and chromosome abnormalities is established. However, since absolute correlations are rare, embryo selection remains one of the most arduous tasks in assisted reproduction. This study was undertaken in order to determine which chromosomal abnormalities are compatible with development to the blastocyst stage. METHODS: Embryos diagnosed by preimplantation genetic diagnosis (PGD) as chromosomally abnormal or unsuitable for transfer were cultured to day 5 or 6. Morphology and development were observed daily. After extended culture, embryos were fixed and analysed by two rounds of FISH with the same probes used for PGD. RESULTS: Some types of numerical chromosome abnormalities do not preclude full differentiation *in vitro*. For instance, extensive mosaicism was detected in blastocysts and trisomic embryos reached the blastocyst stage with a frequency of 37%. Interestingly, only those monosomies compatible with first trimester development (monosomy X and 21) were detected at blastocyst stage. CONCLUSION: Even though there is a strong selection against chromosomally abnormal embryos, extended culture to day 5 or 6 cannot be used as a reliable tool to select against clinically relevant chromosome abnormalities such as trisomies.**

*Key words:* blastocyst/chromosome abnormalities/embryo development/extended culture

## Introduction

Chromosome analysis of human embryos has shown higher rates of aneuploidy than those reported for prenatal testing (Warburton *et al*., 1986; Eiben *et al*., 1994; Jamieson *et al*., 1994), suggesting that considerable numbers of chromosomally abnormal embryos are eliminated early in development. Analysis of oocytes, embryos, spontaneous abortions and live offspring all show an increase in aneuploidy with increasing maternal age (Hassold and Chiu, 1985; Warburton *et al*., 1986; Munné *et al*., 1995; Dailey *et al*., 1996). Embryonic aneuploidy is one of the major causes of reproductive failure in older women, at least after follicular stimulation. Uterine receptivity on the other hand is only marginally affected by age (Navot *et al*., 1994; Munné *et al*., 1995). It has been shown that blastocyst formation after assisted reproduction declines in women aged >30 years, with more arrested embryos at the morula stage (Janny and Ménézo, 1996). We aimed to discover the ability of chromosomally abnormal embryos, diagnosed by fluorescent in-situ hybridization (FISH) on single biopsied cells on day 3, to grow to the blastocyst stage.

Another aim of this study was to determine if culture to blastocyst stage could be an effective tool to select against chromosomally abnormal embryos. Embryo selection can be a powerful tool in establishing high implantation rates. The selection can be pro-active, against morphologically, developmentally or genetically abnormal embryos, or indirect, by culturing the embryos as long as possible. This extra challenge appears to arrest a proportion of unsuitable embryos. Many morphological abnormalities observed between the zygote and cleavage stages have been related to chromosome abnormalities (Munné and Cohen, 1998). However, not all morphologically abnormal embryos are chromosomally affected, and aneuploidy is not associated with morphological abnormalities, at least up to day 3 of development. Therefore, it would be of interest to find a link between blastocyst formation and/or morphology and aneuploidy.

## Materials and methods

Embryos used for this project were donated for research in accordance with guidelines approved by our Internal Review Board, including informed written consent in each case. Embryos were obtained from 31 patients (33 cycles) undergoing preimplantation genetic diagnosis (PGD) for aneuploidy or gender selection for X-linked diseases. Embryos were biopsied on day 3, analysed by FISH, and (i) the chromosomally abnormal embryos or (ii) those without result or (iii) supposedly affected by X-linked diseases were cultured in G2 medium and their development monitored daily until day 5 or 6. Embryo morphology was assessed based on the criteria previously described

**Table I.** Blastocysts and blastomeres analysed

| | |
|---|---|
| Average maternal age (years) | 38.5 |
| Embryos in extended cultured | 254 |
| Embryos analysed | 216 |
| Total no. of nuclei analysed/fixed | 5607/7719 |
| Total no. of blastocysts | 54 |
| Blastocyst formation rate | 21% |
| Blastocysts analysed | 51 |
| Total no. of nuclei analysed/fixed in blastocysts | 3645/5774 |
| Average of nuclei per blastocyst | 113 |
| Average of nuclei analysed per blastocyst | 71 |

(Alikani *et al*., 2000). Embryos classified as arrested were fixed using a published method (Tarkowsky, 1966) while those classified as a blastocyst were fixed according to another protocol (Clouston *et al*., 1997). Chromosomes were analysed with the same probes used in the original PGD analysis. Fixed cells, either for PGD or reanalysis, were tested by two rounds of FISH following the method previously described (Bahçe *et al*., 2000) with the sole modification that the probes were X, Y, 1, 13, 15, 16, 18, 21 and 22.

Published scoring criteria (Munné and Weier, 1996) were used to differentiate FISH errors from mosaicism. To classify the different chromosome abnormalities detected by FISH, we used the following criteria. (i) Normal, aneuploid, haploid and polyploid embryos were those where all the cells of the embryo had the same chromosome constitution or <10% abnormal cells. The 10% threshold is included here because it is the approximate FISH error level with this protocol. (ii) Diploid mosaic embryos were those mosaics that had a diploid line or, on average, the number of chromosomes per cell was diploid. Similarly, when the average number of cells was haploid or polyploid they were classified as haploid and polyploid mosaics respectively. However, to simplify the analysis, polyploid and haploid mosaics were grouped with pure polyploid and haploid embryos. (iii) Some embryos showed chaotic chromosome complements, and, while several authors (Harper *et al*., 1995b; Harper and Delhanty, 1996) consider such embryos as a separated mosaic category, in the present study, these embryos were counted as mosaics following the above classification (diploid, haploid or polyploid mosaics). (iv) Mosaic embryos were sub-classified into extensive and limited mosaics if they had more or less than 38% (3/8) abnormal cells. This definition was applied because of the high frequency of mosaicism in human embryos, which suggests that low doses of mosaicism (limited) may not be detrimental to embryo development (Munné *et al*., 1997). The distinction between extensive and limited mosaicism is not made for haploid and polyploid mosaic embryos because in these, all cells are abnormal by definition. (v) Within the diploid mosaic group, we differentiated those that had a diploid line and a polyploid line (2n/4n mosaics) from the others because the occurrence of polyploid cells in a blastocyst is considered a normal feature of human embryo development (Angell *et al*., 1987; Benkhalifa *et al*., 1993; Drury *et al*., 1998; Evsikov and Verlinsky, 1998) Therefore, if the proportion of polyploid cells was <38%, they were considered normal.

A $\chi^2$-test was applied to compare blastocyst formation rates among the different groups.

## Results

In all, 254 embryos deemed not suitable for transfer after PGD were placed in extended culture. Of those, 216 were reanalysed by FISH on day 5 (Table I). The PGD error rate of false normal and abnormal was 8.6% when day 3 results were compared with those obtained on day 5.

Chromosome abnormalities and blastocyst formation rates are described in Table II. Of the 216 embryos, 15% were chromosomally normal, 38% aneuploid, 38% diploid mosaic, 7% polyploid and 2% haploid, for the chromosomes analysed.

Of the chromosomally normal embryos ($n = 32$), nine were uniformly normal of which three (33%) reached blastocyst stage. However, since not all the cells of the uniformly normal blastocysts were analysed, they could have been 2n/4n mosaics. The other 23 embryos were 2n/4n mosaic with <38% polyploid cells of which 78% developed to blastocyst. On average, 66% of normal embryos reached blastocyst stage.

Of the 83 aneuploid embryos, 23 were monosomics of which only one monosomy 21 and one monosomy X reached the blastocyst stage. Thirty-five trisomies were detected and 37% of those became blastocysts. There were 24 aneuploid embryos that were also extensive mosaics, none of which developed further than the morula stage. The remaining embryo was polyploid mosaic and in addition aneuploid with half the number of chromosomes 21 per cell than the ploidy of each cell (i.e. if the cell was tetraploid, there were two chromosomes 21). Interestingly, this embryo developed to the blastocyst stage. In total, 19% of the aneuploid embryos reached the blastocyst stage, but this low figure is mostly due to arrest of monosomic embryos.

There were 82 diploid mosaics (excluding limited 2n/4n mosaics grouped here with the normal ones) of which nine were extensive 2n/4n mosaics, 33% of which reached blastocyst stage. There were also 24 mosaics with diploid and aneuploid cell lines, and 49 embryos with chaotic cells (Figure 1). Twelve per cent ($n = 6$) of the chaotic mosaics reached blastocyst stage, but only 8% ($n = 2$) of the diploid/aneuploid embryos reached that stage. None of the six chaotic mosaic blastocysts had >60 cells (average: 30 cells, range: 15–51) while the average number of nuclei per blastocyst found in this study was ~114 (5744/51, range: 19–410). Twenty-one per cent of polyploid and none of the haploid embryos developed to blastocyst stage.

In total, 21% (54/254) embryos were classified as blastocyst. However, normal embryos reached blastocyst stage more frequently (66%) than trisomic (37%) ($P = 0.014$), monosomic (9%) ($P = 0.001$), polyploid (21%) ($P = 0.006$), haploid (0%) ($P = 0.01$), and diploid mosaics embryos (13%) ($P < 0.001$) (Table II).

### *Confirmation of PGD diagnosis*

A total of 198 embryos with previous PGD results were reanalysed in day 5. The most serious error classified three embryos as normal by PGD when they should have been considered as abnormal based on the criteria applied in this study. However, all three embryos had a diploid line, being extensive mosaics for chromosome 16, chromosomes 16, 21 and 22 plus polyploidy and finally an extensive mosaicism for polyploidy (considered as an abnormal, and therefore unsuitable for transfer in this study). Fourteen embryos were false abnormal, being seven false monosomies, two false trisomies and five double false aneuploidies. In three of the cases, embryos were limited mosaics for the chromosomes diagnosed

**Table II.** Developmental potential of human embryos depending on their chromosome constitution

| | No. of embryos: | | |
|---|---|---|---|
| | Arrested at day 3 | Arrested at day 4 | Reached blastocyst |
| Normal: 32 (15) | | | |
| 9 uniformly normal 6 | 0 | 3 (33) | |
| 23 mosaic 2n/4n (<38% abnormal) | 3 | 2 | 18 (78)[i] |
| Total | 9 (28) | 2 (6) | 21 (66)[a] |
| Aneuploid: 83 (38) | | | |
| 23 monosomies* | 14 | 7 | 2 (9)‡[b] |
| 35 trisomies† | 15 | 7 | 13 (37)[c] |
| 24 and mosaics (>38% abnormal) | 19 | 5 | 0 (0) |
| 1 and polyploid | 0 | 0 | 1 (100)§ |
| Total | 48 (58) | 19 (23) | 16 (19)[d] |
| Other diploid mosaics: 82 (38) | | | |
| 9 2n/4n (>38% abnormal) | 3 | 3 | 3 (33)[h] |
| 24 2n/aneuploid | | | |
| 6 (10–38% abnormal) | 3 | 2 | 1 (17) |
| 18 (>38% abnormal) | 15 | 2 | 1 (6) |
| 49 2n/chaotic | 36 | 8 | 6 (12) |
| Total | 57 (70) | 15 (18) | 11 (13)[e] |
| Polyploid: 14 (7) | 11 (79) | 0 | 3 (21)[f] |
| Haploid: 5 (2) | 4 (80) | 1 (20) | 0 (0)[g] |

Values in parentheses are percentages.
*Including five embryos with multiple aneuploidy (at least one monosomy).
†Including five embryos with at least trisomy.
‡One monosomy 21, one monosomy X.
§And monosomy 21.
a versus b: $P = 0.001$; a versus c: $P = 0.014$; a versus d, e: $P < 0.001$; a versus f: $P = 0.006$; a versus g: $P = 0.01$; h versus i: $P = 0.022$.

as aneuploid by PGD. The total error rate of false normal and abnormal in this study was 8.6%.

## Discussion

Here it was shown that a small proportion of chromosomally abnormal embryos that cleave for 2 days can develop to the blastocyst stage when cultured *in vitro*. Extended culture is not, however, a reliable selection tool to screen against those chromosomally abnormal embryos that may survive after implantation. Before discussing the ability of chromosomally abnormal embryos to become blastocysts, it is paramount to determine whether embryo biopsy has a detrimental effect on further embryo growth. In this study, the overall blastocyst formation rate was 21%, but for chromosomally normal embryos it was 66%. Although these figures compare favourably with our 45% blastocyst formation rate for non-biopsied embryos (Alikani *et al*., 2000), larger populations need to be studied to confirm these comparisons. Using the present set of results, there seems to be no obvious detrimental effect resulting from embryo biopsy on blastocyst formation, which agrees with other observations (Hardy *et al*., 1990). The ability of embryos to grow to the blastocyst stage *in vitro* should not, however, be equated with developmental competence.

Our data suggest that the modest developmental rates through extended culture are attributable, at least in part, to chromosomal abnormalities. A decrease in embryo survival from cleavage stage to blastocyst is observed in all types of human embryos. Moreover, the results seem to suggest that there is a strong developmental block at compaction, with 65% (120/184) of chromosomally abnormal embryos arresting before compaction, compared with only 28% (9/32) of normal embryos ($\chi^2$-test, $P < 0.001$). The other suggested block, around cavitation (Janny and Ménézo, 1996) was less evident, with 19% (35/184) chromosomally abnormal embryos arresting around morula stage compared with 6% (2/32) of normal embryos (not significant).

The selection against chromosomally abnormal embryos is not the same in all groups. Monosomies, with the exception of monosomy X and 21, haploidies and aneuploidies, combined with extensive mosaicism never developed to blastocyst. Interestingly, the types of chromosome abnormalities observed in blastocysts have a striking resemblance to those found in first trimester conceptuses, that is, trisomies, polyploidy, monosomy X and 21, and limited mosaics (Simoni *et al*., 1986; Simpson, 1990; Wolstenholme, 1996). A recent report by (Evsikov *et al*., 2000) also indicated that embryos with unbalanced translocations, commonly found in first trimester conceptuses, also easily reached blastocyst stage.

The specific arresting stages of unique chromosomal groups require further investigation, particularly as far as developmental anomalies are concerned. For instance, do monosomic embryos arrest when the embryonic genome activates or do they fail to form structural and functional junctions after genomic activation? Of these abnormalities, the least understood is mosaicism. This is a common finding in cleavage-stage human embryos (Munné *et al*., 1994c; Delhanty *et al*., 1997) as well as human blastocysts (Clouston *et al*., 1997; Evsikov and Verlinsky, 1998;

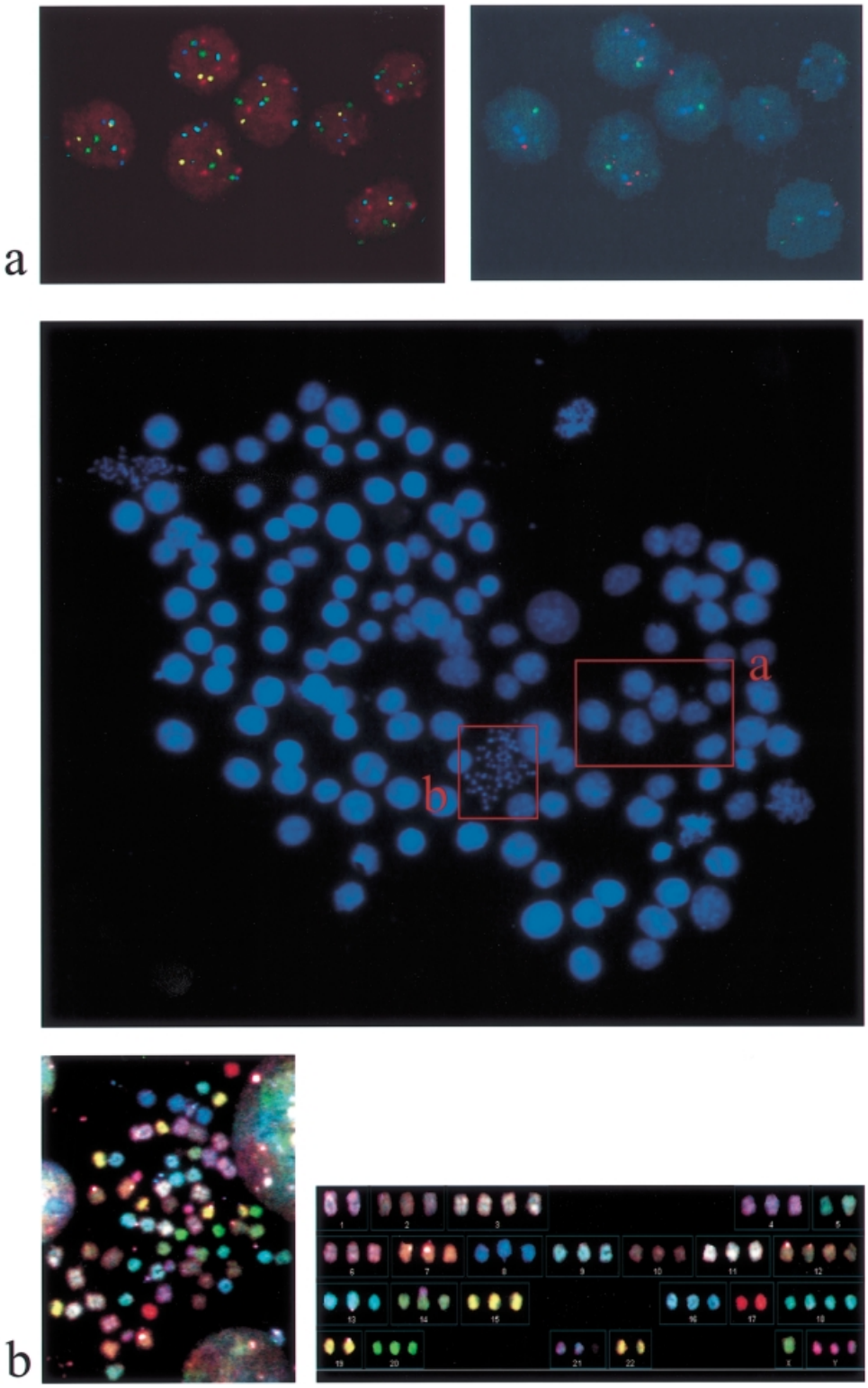


**Figure 1.** Nuclei spread (stained with DAPI) of a mosaic blastocyst. (**a**) Nuclei hybridized in first round with probes for chromosome 13 (red), chromosome 16 (aqua), chromosome 18 (blue), chromosome 21 (green), and chromosome 22 (gold) and second round with probes for chromosome X (green), chromosome Y (orange) and chromosome 15 (aqua). (**b**) Metaphase spread hybridized with SKY technique, showing chaotic karyotype.

Bergers-Janssen *et al*., 1999; Veiga *et al*., 1999; Magli *et al*., 2000; Ruangvutilert *et al*., 2000). We found that 70% of the embryos analysed, independently of the type of ploidy, were mosaics. However, not all types of mosaicism and proportion of abnormal cells have the same impact on embryo development. Although polyploid cells in normal embryos are considered a normal feature of blastocyst formation in all mammalian species studied (Barlow and Sherman, 1972; Hare *et al*., 1980; Long

and Williams, 1982; Murray *et al*., 1986; Angell *et al*., 1987), a high proportion of polyploid cells may be detrimental. In this study, 2n/4n mosaics with <38% abnormal cells developed 78% of the time to blastocyst stage compared with only 33% of those with >38% abnormal cells ($P = 0.021$). Mosaicism due to aneuploid cells seems somehow to interfere with embryo development for reasons that are not entirely clear. Only 11% of diploid mosaic embryos develop to blastocyst, but we did not find statistical differences according to the percentage of abnormal cells (17% for limited versus 6% for extensive mosaicism). However, aneuploidy combined with extensive mosaicism may have a stronger effect as, in this study, none developed to blastocyst. Some chaotic mosaic embryos developed to blastocyst, but were probably developmentally compromised, since they never had more than 60 cells. Though the observations relating blastocyst morphology to chromosomal status are preliminary, we have observed many chromosomally abnormal embryos with abnormalities at the blastocyst stage, including multi-cavitation, extensive exclusion of cells, and an irregularly formed trophectoderm.

In conclusion, while an early developmental block seems to prevent full differentiation of chromosomally abnormal embryos, extended culture does not universally select against all anomalies. The data also imply that the decrease in implantation with advanced maternal age is partially due to the arrest of chromosomally abnormal embryos prior to blastocyst formation.

## Acknowledgements

The authors gratefully acknowledge the efforts of the team of embryologists at the Institute for Reproductive Medicine and Science of Saint Barnabas Medical Center for their support of this study. Our thanks to Giles Tomkin for editorial assistance in the preparation of this manuscript.

## References

Alikani, M., Calderon, G., Tomkin, G. *et al*. (2000) Cleavage anomalies in early human embryos and survival after prolonged culture in-vitro. *Hum. Reprod*., **15**, 2634–2643.

Angell, R.R., Sumner, A.T., West, J.D. *et al*. (1987) Post-fertilization polyploidy in human preimplantation embryos fertilized *in vitro*. *Hum. Reprod*., **2**, 721–727.

Bahçe, M., Escudero, T., Sandalinas, M. *et al*. (2000) Improvements of preimplantation diagnosis of aneuploidy by using microwave-hybridization, cell recycling and monocolor labeling of probes. *Mol. Hum. Reprod*., **6**, 849–854.

Barlow, P.W. and Sherman, M.I. (1972) The biochemistry of differentiation of mouse trophoblast: studies on polyploidy. *J. Embryol. Exp. Morphol*., **27**, 447–465.

Benkhalifa, M., Janny, L., Vye, P. *et al*. (1993) Assessment of polyploidy in human morulae and blastocysts using co-culture and fluorescent in-situ hybridization. *Hum. Reprod*., **8**, 895–902.

Bergers-Janssen, J.M., Derhaag, J.G., Ignoul-Vanvuchelen, R.C.M. *et al*. (1999) Inventory of chromosome abnormalities in human blastocysts using multiple probes and repeated fluorescence in-situ hibridization. In *Proceedings of the European Society of Human Reproduction and Embryology*, Tours, France, June 1999, *Hum. Reprod.*, **14** (Abstract Bk.1), O-137, p. 75.

Clouston, H., Fenwick, J., Webb, A. *et al*. (1997) Detection of mosaic and non mosaic chromosome abnormalities in 6 to 8 day-old human blastocysts. *Hum. Genet*., **101**, 30–36.

Dailey, T., Dale, B., Cohen, J. *et al*. (1996) Association between non-disjunction and maternal age in meiosis-II human oocytes detected by FISH analysis. *Am. J. Hum. Genet*., **59**, 176–184.

Delhanty, J.D.A., Harper, J.C., Ao, A. *et al*. (1997) Multicolour FISH detects frequent chromosomal mosaicism and chaotic division in normal preimplantation embryos from fertile patients. *Hum. Genet*., **99**, 755–760.

Drury, K., Kovalinskaia, R. and Williams, S. (1998) Polyploidy as a normal function of trophoblastic development in human preimplantation embryos observed by fluorescent *in situ* hybridization analysis. *Fertil. Steril*., **70** (Suppl.), 10–25.

Eiben, B., Goebel, R., Hansen, S. *et al*. (1994) Early amniocentesis. A cytogenetic evaluation of over 1500 cases. *Prenat. Diagn*., **14**, 497–501.

Evsikov, S. and Verlinsky, Y. (1998) Mosaicism in the inner cell mass of human blastocysts. *Hum. Reprod*., **11**, 3151–3155.

Evsikov, S., Cieslak, M.L.T. and Verlinsky, Y. (2000) Effect of chromosomal translocations on the development of preimplantation human embryos *in vitro*. *Fertil. Steril*., **74**, 672–677.

Hardy, K., Martin, K.L., Leese, H.J. *et al*. (1990) Human preimplantation development *in vitro* is not adversely affected by biopsy at the 8-cell stage. *Hum. Reprod*., **5**, 708–714.

Hare, W.C.D, Singh, E.L., Betteridge, K.J. *et al*. (1980) Chromosomal analysis of 159 bovine embryos collected 12 to 18 days after estrus. *Can. J. Genet. Cytol*., **22**, 615–626.

Harper, J.C. and Delhanty, J.D.A. (1996) Detection of chromosomal abnormalities in human preimplantation embryos using FISH. *J. Assist. Reprod. Genet*., **13**, 137–139.

Harper, J.C., Drawson, K., Delhanty, J.D.A. *et al*. (1995) The use of fluorescent in-situ hybridization (FISH) for the analysis of in-vitro fertilization embryos: a diagnostic tool for the infertile couple. *Hum. Reprod*., **10**, 3255–3258.

Hassold, T. and Chiu, D. (1985) Maternal age-specific rates of numerical chromosome abnormalities with special reference to trisomy. *Hum. Genet*., **70**, 11–17.

Jamieson, M.E., Coutts, J.R.T. and Connor, J.M. (1994) The chromosome constitution of human preimplantation embryos fertilized *in vitro*. *Hum. Reprod*., **9**, 709–715.

Janny, L. and Ménézo, Y.J.R. (1996) Maternal age effect on early human embryonic development and blastocyst formation. *Mol. Reprod. Dev*., **45**, 31–37.

Long, S.E. and Williams, C.V. (1982) A comparison of the chromosome complement of inner cell mass and trophoblast cells in day-10 pig embryos. *J. Reprod. Fertil*., **66**, 645–648

Magli, M.C., Jones, G.M., Gras, L. *et al*. (2000) Chormosome mosaicism in day 3 aneuploid embryos that develop to morphologically normal blasstocysts *in vitro*. *Hum. Reprod*., **15**, 1781–1786

Munné, S. and Weier, U. (1996) Simultaneous enumeration of chromosomes 13, 18, 21, X and Y in interphase cells for preimplantation genetic diagnosis of aneuploidy. *Cytogenet. Cell Genet*., **75**, 263–270.

Munné, S. and Cohen, J. (1998) Chromosome abnormalities in human embryos. *Hum. Reprod. Update*, **4**, 842–855.

Munné, S., Weier, H.U.G., Grifo, J. *et al*. (1994) Chromosome mosaicism in human embryos. *Biol. Reprod*., **51**, 373–379.

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

Munné, S., Magli, C., Adler, A. *et al*. (1997) Treatment-related chromosome abnormalities in human embryos. *Hum. Reprod*., **12**, 780–784.

Murray, J.D., Moran, C., Boland, M.P. *et al*. (1986) Polyploid cells in blastocysts and early fetuses from Australian Merino sheep. *J. Reprod. Fertil*., **78**, 439–446.

Navot, D., Drews, M.R., Bergh, P.A *et al*. (1994) Age related decline in female fertility is not due to diminished capacity of the uterus to sustain embryo implantation. *Fertil. Steril*., **61**, 97–101.

Ruangvutilert, P., Delhanty, J.D.A., Serhal, P. *et al*. (2000) FISH analysis on day 5 post-insemination of human arrested and blastocyst stage embryos. *Prenat. Diagn*., **20**, 552–560.

Simoni, G., Gimelli, G., Cuoco, C. *et al*. (1986) First trimester fetal karyotyping: one thousand diagnoses. *Hum. Genet*., **72**, 203–209.

Simpson, J.L. (1990) Incidence and timing of pregnancy losses: relevance to evaluating safety of early prenatal diagnosis. *Am. J. Med. Genet*., **35**, 165–173.

Tarkowski, A.K. (1966) An air drying method for chromosome preparations from mouse eggs. *Cytogenetics*, **5**, 394–400.

Veiga, A., Gil, Y., Boada, M. *et al*. (1999) Confirmation of diagnosis in preimplantation genetic diagnosis (PGD) through blastocyst culture: preliminary experience. *Prenat. Diagn*., **19**, 1252–1247.

Warburton, D., Kline, J., Stein, Z. *et al*. (1986) Cytogenetic abnormalities in spontaneous abortions of recognized conceptions. In Porter, I.H. and Willey, A. (eds), *Perinatal Genetics: Diagnosis and Treatment*. Academic Press, New York, pp. 133–148.

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.

*Received on November 6, 2000; accepted on May 31, 2001*