Trisomy 1 in an eight cell human pre-embryo

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SUMMARY The high incidence of chromosome abnormalities in clinically recognised pregnancies is well documented, but experience of these problems at the time of conception is extremely limited. Using donated oocytes from women seeking surgical sterilisation, we have established reliable cytogenetic techniques for chromosome analysis of human pre-embryos. These have resulted in the first report of trisomy 1. The pre-embryo showed no other obvious abnormality in relation to follicular characteristics, embryo morphology, and cleavage kinetics. The usefulness of such data in explaining the high incidence of occult human pregnancy loss and the current poor success following embryo replacement is emphasised.

It is generally accepted that chromosome abnormalities are a major cause of spontaneous abortion in man,1 with more than half of the first trimester spontaneous abortions being chromosomally abnormal.2 3 Triploidy is common, as is monosomy X, while trisomy for 21 of the 22 human autosomes has been reported. Trisomy 5 is very rare4 and, to the best of our knowledge, trisomy 1 has not been previously documented.

The recent advent of in vitro fertilisation (IVF) has provided a unique opportunity to study chromosomes at the earliest embryonic stages. The supply of pre-embryos is scant and cytogenetic study has been limited to the first few cleavage divisions. Thus, chromosome harvesting techniques have been fraught with problems in view of the small number of cells and the difficulty in estimating when metaphase is due to occur in the cell cycle. However, despite these technical difficulties, a few reports of the chromosome constitution in IVF pre-embryos are beginning to appear, detailing aneuploid conditions rarely seen in clinically recognised pregnancies, for example, monosomy and nullisomy.5

We would like to augment this very limited body of knowledge by reporting a case of trisomy 1 in an eight cell human pre-embryo arising through IVF of a volunteer donor oocyte.

Material and methods

A previously described6 7 schedule for ovulation induction and follicle aspiration was used in a volunteer woman requesting laparoscopic sterilisation. This technique results in an average recovery of 2.1 oocytes per patient and the current fertilisation rate is 67%. The clinical and laboratory techniques have the approval of the Joint Ethical Committee of the Grampian Health Board and the University of Aberdeen.

Insemination is carried out using fertile cryostored donor sperm. The fertilisation and culture techniques are standard.5 6

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FIG 1 Scale diagram of Petri dish with the three drops of solutions used in harvesting procedure, that is, (1) Dispase, (2) KCl, (3) distilled water.
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Pre-embryos are examined in detail and graded on a scale of 0 to 10 according to recommended morphological criteria.8 The stage of the cell cycle is estimated using the cleavage history as a guide. Colcemid (0.5 μg/ml) is added at a suitable time in an attempt to arrest the blastomeres at the metaphase of mitosis. This is not always possible since the zona pellucida appears to prevent the entry of colcemid in some cases and there have been several recordings of cleavage in the presence of quite high doses of colcemid.

The pre-embryos are harvested by a modified cytogenetic method suitable for small amounts of cells. This is done using an inverted microscope and hand held micropipettes (a fresh one for each stage) made from the drawn out tip of a pasteur pipette with a small silicone tubing bulb, plugged with a tiny piece of glass. These pipettes must be tested before use and ragged glass ends (seen only under the microscope) must be heat sealed to prevent the pre-embryo from being damaged.

The harvesting technique is a modification of that of Angell et al.5 The pre-embryo is moved through small drops of three solutions kept in a 2.5 cm culture dish, which are labelled with alcohol based felt pen on the outside of the dish (fig 1).

The pre-embryo is observed during each transfer and it is essential that a minimum amount of each solution is transferred to the next drop with the embryo. The solutions are: (1) one drop Dispase (4 mg/ml) (Boeringer) for five minutes; (2) one drop KCl hypotonic (1%) for five minutes; and (3) one drop distilled water for one minute (critical).

The pre-embryo is then transferred to a pre-cleaned slide marked on the back with a small circle. A microdrop of water, hopefully containing the cells, is placed within the circle and a small line of microdrops can be placed to either side (fig 2). Microscopic examination will verify the position of the embryo, the spare drops can be wiped off, and the circle re-sited if necessary. The amount of water surrounding the embryo is critical and must be as small as possible to minimise fixation and minimise spread.

A single drop of 3 methanol:1 glacial acetic acid fix is then dropped straight onto the pre-embryo from one inch and several more drops of fix are added from the side so that the fix washes over the cells allowing them to ‘almost dry’ between applications. The cells are watched during this entire procedure and can be examined under phase when dry for the presence of chromosomes. The present success rate in obtaining chromosomes is around 25% in this laboratory.

In the case presented here, the chromosomes were stained with Giemsa, photographed, destained, sequentially C banded, and re-photographed.

Results

The pre-embryo was given a grading of 10, since it had even and rapid cleavage (eight cells more than 72

FIG 2 Scale diagram of slide with line of microdrops, the one containing the blastomeres being circled.

FIG 3 Eight cell human pre-embryo, grade 10.
FIG 4  (a) Karyotype stained with Giemsa illustrating the 47,XY,+1. (b) C banded metaphase verifying the trisomy 1 and the Y.
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hours after fertilisation), no granules, and no nuclear fragmentation (fig 3). Giemsa staining, verified by C banding, showed a trisomy 1 male mitotic karyotype (fig 4). This was verified in a second early metaphase cell, while the other six nuclei were out of synchrony in interphase.

Parental C banded chromosomes suggested that the extra chromosome 1 originated in a viable sperm from the father and that non-disjunction at the second meiotic division was the most likely explanation (fig 5).

Discussion

The morphology and cleavage kinetics for this embryo were apparently normal, so there would have been no reason to exclude it from embryo replacement, had it been intended for this purpose.

It is clear that chromosome analysis at conception will reveal a greater range of gross aberrations than has been reported in first trimester spontaneous abortions. Many of these abnormalities are probably incapable of even rudimentary fetal development. These would include trisomy 1 and many monosomies. This is not entirely surprising, since it is known that other trisomies may be compatible with only limited development of fetal membranes but not of a fetus itself, for example, trisomy 2, and only very few trisomic conditions are found in term neonates.

The lack of reports of trisomy 1 in clinically recognised pregnancies may have been due to gamete selection, since gametes carrying two chromosomes 1 may have been inviable or incapable of fertilisation. This is clearly not the case in vitro although in vivo gamete selection remains a possibility.

The most likely explanation is that a trisomy 1 embryo is capable of normal early cleavage, but is probably not compatible with implantation.

It is anticipated that further studies of this nature will give vital information regarding the low success rate following embryo replacement in IVF programmes, as well as elucidating the nature and incidence of chromosome abnormalities at conception.

References

1 Warburton D, Stein Z, Kline J, Susser M. Chromosome abnormalities in spontaneous abortion: data from the New


Some notes for contributors on nomenclature

Nomenclature. Authors should refer to the following publications.


Trisomy 1 in an eight cell human pre-embryo.

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