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Chromosome abnormalities detected in chorionic villus biopsies of failing pregnancies in a subfertile population

ADRIAN M. LOWER, MARIE T. MULCAHY, JOHN L. YOVICH

Abstract

Objective—To determine the range and prevalence of chromosomal abnormalities occurring in failing pregnancies in subfertile women.
Design—Prospective biochemical and ultrasound monitoring of all pregnancies conceived between 1988 and 1990 in a subfertile population.
Setting—A single-centre specialist fertility clinic in Perth, Western Australia.
Subjects—Tissue from 50 early pregnancy losses was successfully cultured for chromosomal analysis from 46 pregnancies comprising 29 anembryonic pregnancies, 9 miscarriages and 8 ectopic pregnancies.
Main outcome measures—Impending pregnancy loss was identified at an early stage. Chromosomal analysis was performed on chorionic villi obtained before the diagnosis became clinically evident.
Results—Significant chromosomal abnormalities were identified in 54% (14/26) of early pregnancy losses where gamete manipulation was involved and 45% (9/20) of those following spontaneous conception. The most common abnormalities were trisomies (12 pregnancies, mainly trisomy 16), triploidies (3 pregnancies) and monosomy X (3 pregnancies). An excess of female fetuses was noted with only 24% of conceptuses (11/46) bearing a Y chromosome.
Conclusions—The data indicate a similar rate of chromosomal abnormalities underlying pregnancy losses at earlier stages of pregnancy and after infertility treatments as that reported from the general population. Gamete manipulation does not appear to confer a higher rate of chromosomal abnormalities in ensuing pregnancies.

The diagnosis of pregnancy in women who conceive after subfertility should be received with cautious optimism as early pregnancy wastage is high (25–30%) and includes a 5–6% risk of ectopic pregnancy (Yovich & Matson 1988; National Perinatal Statistics Unit 1990). The underlying causes of pregnancy wastage are likely to be related to the same factors responsible for implantation disorders, namely embryo quality and uterine receptivity (Yovich & Lower 1990). A major contribution to embryo abnormality undoubtedly arises from underlying chromosomal defects. Previous studies have identified chromosome abnormalities in approximately 50% of the products of conception which were karyotyped after miscarriage (Boue *et al.* 1975; Hassold *et al.* 1980b; Byrne *et al.* 1985). These

The London Hospital Joint Academic Unit, White Chapel, London, UK
A. M. LOWER

Cytogenetics Unit, State Health Laboratories, Perth, Western Australia
M. T. MULCAHY

PIVET Medical Centre, Perth, Western Australia
J. L. YOVICH

Correspondence: J. L. Yovich, PIVET Medical Centre, 166 Cambridge Street, Leederville, Perth, Western Australia.

studies were performed in the general population and examined the products of conception after the diagnosis had been made on clinical grounds, in most cases after miscarriage had occurred. Since there is a high rate of pregnancy wastage in women who conceive after assisted conception, monitoring of serum hormone estimations and serial ultrasound examination is offered to women at PIVET Medical Centre during the first trimester. This has enabled failing pregnancies to be identified before the diagnosis becomes clinically obvious (Yovich *et al.* 1986). Chorionic villus samples have thus been obtained at an earlier stage of gestation providing a prospective study of all failing pregnancies which occurred in a subfertile population from a single centre.

Subjects and methods

Subfertile women conceiving at PIVET Medical Centre as a result of assisted conception procedures, artificial insemination or timed intercourse were monitored through the luteal phase and the first trimester by serial estimations of serum levels of the β-subunit of human chorionic gonadotrophin (β-hCG), oestradiol and progesterone, with ultrasound examination at 7 weeks gestation (dated from the last menstrual period (LMP) or adjusted LMP) or earlier if indicated. Early pregnancy losses were diagnosed according to the following criteria.

Blighted ovum (Anembryonic pregnancy)

In 30 women early ultrasound examination revealed an empty intrauterine gestation sac associated with static or falling serum β-hCG levels. Mean gestational age at sampling was 7·72 weeks (range 7–10 weeks).

Spontaneous fetal death (miscarriage)

Eleven women had a diagnosis of absent fetal heart activity and failure in growth of the gestation sac, when positive fetal heart activity had previously been diagnosed at ultrasound scan. Mean gestational age at sampling was 10·5 weeks (range 8–17 weeks).

Ectopic pregnancy

This was strongly suspected in nine women when ultrasound examination failed to confirm the presence of an intrauterine gestation sac in association with raised serum β-hCG. The diagnosis was confirmed at laparoscopy or laparotomy. Mean gestational age at sampling was 7·44 weeks (range 6–8 weeks).

Chorionic villus sampling

Chorionic villi were obtained at the time of but before surgical termination of pregnancy using a malleable Birmingham transcervical aluminium catheter (Rocket, London). Specimens were immediately examined in the adjacent IVF laboratory at ×40 magnification using an inverted microscope. Villi were dissected free from any contaminating maternal blood and tissue by the embryologist before being placed in a Tyrodes based solution with Hepes buffer (Quinn *et al.* 1985) and transported to the cytogenetics laboratory. There the specimen was again inspected using an inverted microscope and pieces of villi were selected for culture. These were washed several times in phosphate buffered saline and dissected free of all maternal tissue. When readily identifiable villi could not be separated, a portion of membraneous tissue was selected and cleaned but the specimen was classified as products of conception rather than confirmed villi.

The specimen was then minced and cultured in Chang's medium in 5% CO_2 at 37°C. When cultures were confluent they were trypsinized and harvested subsequently by the suspension method after exposure to Colcemid for approximately 3 h, followed by hypotonic treatment and fixation in 3 : 1 methanol/acetic acid. A minimum of 15 cells were analyzed from each specimen. Identification of chromosomes was made using GTG banding (Seabright 1971) (Fig. 1).

Results

Over a 2-year period 50 pregnancies were examined. Four have been excluded because of failed culture. Of the remaining 46 pregnancies there were 29 blighted ova, 9 miscarriages and 8 ectopic pregnancies. In 10 of the 29 blighted ovum pregnancies and in 1 of the 9 miscarriages readily identifiable villi could not be unequivocally separated and the culture specimen, consisting of membraneous tissue, was classified as products of conception. These 11 specimens all gave normal 46XX karyotypes. Whilst most of these karyotypes are thought to be of fetal origin, it

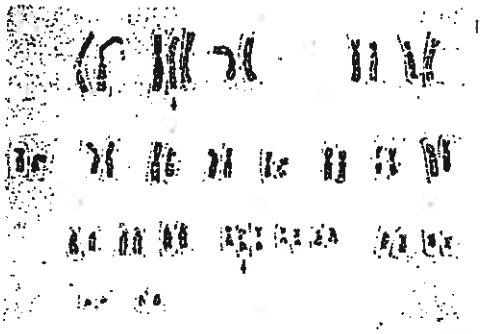

Fig. 1. Human karyotype obtained using GTG banding. 48XX+2+16.

was not possible to confirm conclusively that some were not of maternal origin.

Table 1 summarises the number and types of chromosomal abnormalities obtained in conceptions with respect to gamete manipulation. The proportion of abnormalities in early pregnancy losses following gamete manipulation (14/26; 54%) is not significantly different from those following natural conception (9/20; 45%). The results are presented graphically in Fig. 2. Anembryonic pregnancies and miscarriages revealed a higher proportion of chromosomal abnormalities than ectopic pregnancies.

There was no statistically significant difference in the maternal age between the women who experienced an early pregnancy loss with a normal karyotype and those with an abnormal karyotype (mean age 32·2 years, SD 4·3 *vs* mean 32·8 years, SD 4·7).

Discussion

As the laboratory techniques associated with assisted conception have improved, and particularly with the introduction of new procedures for the treatment of male infertility, the fertilization rate of oocytes following IVF has risen to around 80%. This compares favourably with an estimation of 84% of human ova exposed to the opportunity of fertilization *in vivo* (Leridon 1977). However, the clinical pregnancy rate is still relatively low, diminished in part by failure to implant and partly from preclinical losses (Yovich & Matson 1988). Chromosomal abnormalities have been demonstrated in 40% of preimplantation embryos generated following IVF (Papadopoulos *et al.* 1989) and have been shown to be by far the most common identifiable defect among early pregnancy losses occurring after implantation. One might speculate that other aetiological factors peculiar to pregnancies conceived following IVF could have an adverse effect on the rate of early pregnancy loss. Our results do not confirm this, with approximately 50% being affected by chromosomal abnormalities. Furthermore in a study conducted by postal questionnaire of more than 200 European IVF centres, 21 of 34 (62%) fetal losses karyotyped demonstrated a chromosomal abnormality (Plachot 1989). One small study from a single assisted conception unit reported 5 abnormalities out of 13 successfully karyotyped miscarried fetuses (Roesler *et al.* 1989). Other studies, not confined to the subfertile populations, also encountered abnormalities in around 50% of lost fetuses (Boue *et al.* 1975). Data pooled from several series reveals an overall prevalence of chromosomal abnormality in 46% of miscarriages recognized in the first trimester (Simpson & Bombard 1987). The most common anomaly was autosomal trisomy accounting for 50% of all abnormalities detected and 22% of all miscarriages for which karyotypes were available. A more recent study has demonstrated an even higher proportion of abnormalities (76·7%) although a number of these women had been referred for chorionic villus sampling because of increased risk of fetal chromosomal abnormality (Guerneri *et al.* 1987).

The relation between maternal age and aneuploidy is well established, in particular for trisomy 21 (Penrose, 1933). Our findings were not influenced by age-related effects since no significant difference was noted in maternal age between women losing normal and abnormal conceptuses.

Our findings indicate a male karyotype is less likely to miscarry. There was not a single normal

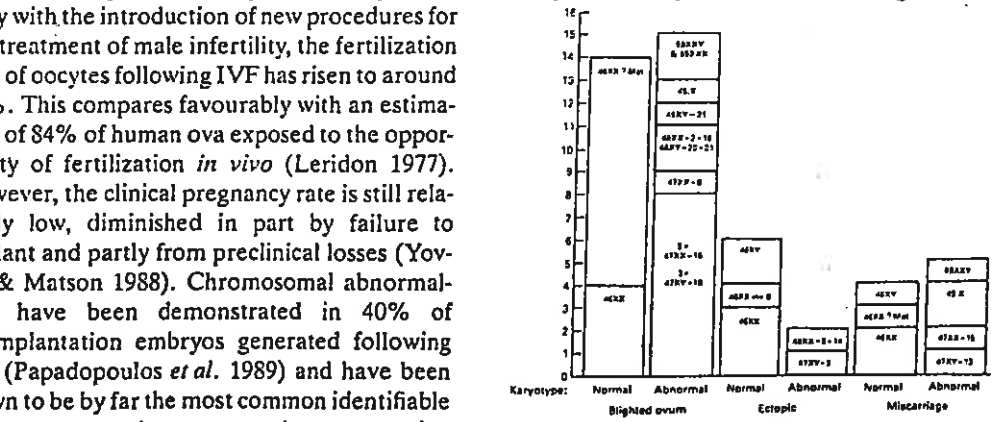

Fig. 2. Distribution and types of chromosomal abnormalities.

Table 1. Karyotyping of early pregnancy losses in 46 subfertile women

Subject No.	Age (years)	Stimulation protocol	Procedure	Diagnosis	Tissue examined	Karyotype
Gamete manipulation						
1	40	Lucrin/hMG	IVF-ET	Bl ovum	Conf villi	47XX+16
30	37	Lucrin/hMG	IVF-ET	Ectopic	Conf villi	47XY+20
24	32	Lucrin/hMG	IVF-ET	Bl ovum	POC	46XX
2	26	Lucrin/hMG	PROST	Bl ovum	Conf villi	48XX+2+16
38	31	Lucrin/hMG	PROST	Miscarriage	Conf villi	45,X
3	36	Lucrin/hMG	TEST	Bl ovum	Conf villi	47XX+16 inv 2
4	29	Lucrin/hMG	TEST	Bl ovum	Conf villi	45XY−21
31	38	Lucrin/hMG	TEST	Ectopic	Conf villi	48XX+8+14
40	34	Lucrin/hMG	TEST	Miscarriage	Conf villi	47XY+13
16	32	Lucrin/hMG	TEST	Bl ovum	Conf villi	46XX
20	33	Lucrin/hMG	TEST	Bl ovum	POC	46XX
21	38	Lucrin/hMG	TEST	Bl ovum	POC	46XX
22	38	Lucrin/hMG	TEST	Bl ovum	POC	46XX
19	29	hCG	FROST	Bl ovum	Conf villi	46XX
7	33	Nil	FET	Bl ovum	Conf villi	47XY+16
34	34	Nil	FET	Ectopic	Conf villi	46XY
41	38	Lucrin/hMG	GIFT	Miscarriage	Conf villi	47XX+15
39	30	Lucrin/hMG	GIFT	Miscarriage	Conf villi	45,X
32	33	Lucrin/hMG	GIFT	Ectopic	Conf villi	46XX inv 6
23	41	Lucrin/hMG	GIFT	Bl ovum	POC	46XX
45	41	HRT	ET OD	Miscarriage	Conf villi	46XX
5	34	hMG	DI	Bl ovum	Conf villi	47XX+16
6	41	hMG	i/c	Bl ovum	Conf villi	48XY+20+21
46	30	hMG	i/c	Miscarriage	POC	46XX
17	28	CC/hMG	i/c	Bl ovum	Conf villi	46XX
18	29	CC	i/c	Bl ovum	Conf villi	46XX (Twins)
Natural conception						
9	26	Nil	i/c	Bl ovum	Conf villi	47XX+16
10	37	Nil	i/c	Bl ovum	Conf villi	47XX+16
11	30	Nil	i/c	Bl ovum	Conf villi	47XY+16
13	38	Nil	i/c	Bl ovum	Conf villi	47XY+16
14	31	Nil	i/c	Bl ovum	Conf villi	47XX+6
8	27	Nil	i/c	Bl ovum	Conf villi	69XXY
12	31	Nil	i/c	Bl ovum	Conf villi	69XXX
42	31	Nil	i/c	Miscarriage	Conf villi	69XXY
15	24	Nil	i/c	Bl ovum	Conf villi	45,X
33	29	Nil	i/c	Ectopic	Conf villi	46XY
44	37	Nil	i/c	Miscarriage	Conf villi	46XY
35	27	Nil	i/c	Ectopic	Conf villi	46XX
36	33	Nil	i/c	Ectopic	Conf villi	46XX
37	31	Nil	i/c	Ectopic	Conf villi	46XX
43	27	Nil	i/c	Miscarriage	Conf villi	46XX
25	31	Nil	i/c	Bl ovum	POC	46XX
26	35	Nil	i/c	Bl ovum	POC	46XX
27	27	Nil	i/c	Bl ovum	POC	46XX
28	28	Nil	i/c	Bl ovum	POC	46XX
29	31	Nil	i/c	Bl ovum	POC	46XX

Abbreviations. Lucrin = Leuprolide Acetate (GnRH Analog); hMG = human menopausal gonadotrophin; HRT = hormone replacement therapy; CC = clomiphene citrate; IVF-ET = in vitro fertilization and embryo transfer; PROST = pronuclear stage tubal transfer; TEST = tubal embryo stage transfer; FROST = frozen/thawed embryo salpingeal transfer; FET = frozen embryo transfer; GIFT = gamete intrafallopian transfer; ET OD = intrauterine embryo transfer donated ooctye; DI = Donor insemination; i/c = timed/natural intercourse; Conf villi = confirmed villi on culture; POC = culture of villi not confirmed.

male karyotype among the blighted ovum pregnancies which constitute the major proportion of all pregnancy failures. Furthermore, only 24% of all conceptuses karyotyped bore a Y chromosome, and only 13% of the conceptuses with apparently normal karyotypes were male. Even if the 11 pregnancies in which definite villi were not identified are excluded, only 25% of normals were male. Data from the National Perinatal Statistics Unit in Australia and New Zealand where the overall sex ratio in singleton births following IVF procedures in 1988 was 1·03 has confirmed that gamete manipulation cannot be held responsible for such variation between the sexes (National Perinatal Statistics Unit 1990). An excess of female miscarried fetuses over males was also noted in the two largest reported series of miscarriages (Boue *et al.* 1975; Creasy *et al.* 1976). A more recent report from Germany of 750 miscarriages also demonstrated an excess of female karyotypes in chromosomally normal fetuses (male : female ratio 0·71) (Eiben *et al.* 1990). Since it has already been acknowledged that the sex ratio approaches unity at birth, the data suggest that more female embryos must either fertilize or implant.

Knowledge of the karyotype may be of relevance to the prognosis in subsequent pregnancies. An abnormal chromosome complement was found in 80% of pregnancies in women whose previous pregnancy resulted in miscarriage with chromosomal abnormalities (Hassold 1980a). In other words certain couples seem to be predisposed to recurrent aneuploidy. Whilst this is often manifest as recurrent miscarriage, several studies have indicated that the risk of liveborn trisomy 21 following miscarriage of a trisomic pregnancy is about 1% (Alberman 1981). Therefore consideration should be given to prenatal diagnosis in such women.

Two of the three triploid karyotypes identified are of particular interest as they occurred in consecutive naturally conceived pregnancies in the same woman. Whether these lesions were the result of polyspermic fertilization or caused by a defect of cell division is unknown. Controlled IVF is proposed as a diagnostic test for this couple. If polyspermic fertilization is demonstrated at the pronuclear stage then the condition may be amenable to specific treatment by microinjection of a single spermatozoon if the technique of micromanipulation continues to develop from its present stage (Ng *et al.* 1988). The importance of careful examination of the fertilised oocytes at the pronuclear stage has been stressed previously (Yovich, 1985). In particular this will allow oocytes with odd numbers of pronuclei to be discarded. There were no cases of polyploidy amongst those pregnancies conceived following IVF in the present series.

In summary, early diagnosis of pregnancy failure has enabled accurate karyotyping in a high proportion of the early pregnancy losses arising after treatment in this clinic. The results are largely in agreement with the published series of chromosomal analysis of the products of conception obtained following miscarriage in the fertile population; a similar range and distribution of karyotypes were obtained.

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