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Cytogenetic analysis of human oocytes and embryos in an in-vitro fertilization programme

W.R.Edirisinghe[1,2], A.R.Murch[3] and J.L.Yovich[1,4]

[1]PIVET Medical Centre, Cambridge Street, Leederville (Perth) 6007, Western Australia

[2]Present address: National University of Singapore, Lower Kent Ridge Road, Singapore 0511

[3]Present address: Combined Clinical Cytogenetics Unit, Queen Elizabeth II Medical Centre, Nedlands (Perth) 6009, Western Australia

[4]To whom correspondence should be addressed

Oocytes (unfertilized and preovulatory) and embryos (normal and polypronuclear), which were donated to research by patients undergoing procedures of assisted reproductive treatment, were analysed for cytogenetic abnormalities. A total of 362 oocytes and embryos were analysed. The unfertilized oocytes with readable metaphases (53.4%) gave 25.2% chromosomal abnormality with diploidy being the main aberration observed. A high incidence of premature chromosome condensation (PCC) was observed and the incidence of PCC in oocytes exposed to colcemid was significantly higher (14/62, 22.6%) than in those not exposed to this treatment (3/41, 7.3%, $P < 0.05$). When chromosomal anomalies and PCC in the unfertilized oocytes were correlated to various patient criteria such as stimulation regimen, number of human menopausal gonadotrophin ampoules, peak oestradiol levels, age of patient and number of previous attempts, none of the criteria tested had any significant relationship to the incidence of chromosomal abnormality. However a significant increase in the incidence of PCC was noted in the gonadotrophin-releasing hormone (GnRH) 'flare' group (6/15, 40.0%) compared to the GnRH 'down-regulation' group (11/88, 12.5%). The incidence of chromosomal abnormalities among preovulatory oocytes was 16.7% and diploidy was the only abnormality noted. For embryos arising from two-pronuclear oocytes, the chromosomal constitution related mainly to embryo quality. The rate of chromosomal abnormality for apparently good quality embryos was 23.5% and for poor or fragmented embryos 83.3%. The majority (77.3%) of the readable metaphase plates for polypronuclear 1-cell and cleaved embryos showed grossly abnormal chromosome complements but 19% of the cleaved embryos contained sets of normal diploid chromosomes. The chromosomal abnormalities observed among oocytes and embryos are discussed in relation to similar material analysed by other in-vitro fertilization (IVF) programmes. The relevance with respect to assessing IVF techniques, patient factors and improvement of culture conditions is considered.

Key words: chromosome abnormality/embryos/human IVF/oocytes

Introduction

In-vitro fertilization (IVF) and related techniques have become an established treatment for certain forms of human infertility. However, a high rate of pregnancy loss is observed following assisted reproduction (Seppälä, 1985; Yovich and Matson, 1988). Cytogenetic analysis of these abortuses has indicated a high incidence of chromosomal abnormality (Plachot, 1989; Yovich and Lower, 1991). Even in spontaneous abortions in natural conception cycles, ~60% carry a chromosomal anomaly (Boué *et al.*, 1975) and the main chromosomal aberration identified in the abortuses is aneuploidy (Hassold *et al.*, 1980).

Introduction of IVF as an infertility treatment has made possible the study of early events in reproduction and the contribution of oogenesis, fertilization or early embryogenesis to the incidence of chromosomal abnormalities. Several groups have attempted to analyse the chromosome complement, showing the incidence of chromosomal aberrations in the human oocyte to range from 11% (Spielmann *et al.*, 1985) to 22% (Plachot *et al.*, 1986) and 23% (de Jong *et al.*, 1985) with a similar or higher incidence for cleaved human embryos (e.g. 40%, Papadopoulos *et al.*, 1989).

Various steps in IVF procedures, such as ovarian stimulation, oocyte aspiration, culture of oocytes *in vitro*, insemination with spermatozoa of varying concentrations and quality and culture of cleaving embryos *in vitro*, may all contribute to the final chromosome constitution of the embryos. Furthermore, subtle differences in each of these steps may contribute to variations in the incidence of chromosome abnormalities observed among different study groups.

Many superovulatory drugs are used in IVF programmes. Recently, with the use of gonadotrophin-releasing hormone (GnRH) analogues and human menopausal gonadotrophin (HMG), increased numbers of oocytes are recovered with associated high peak oestradiol levels (Cummins *et al.*, 1989). Embryos resulting from cycles stimulated with HMG alone have shown a low incidence of chromosomal abnormality, mainly triploidy, compared to clomiphene/HMG cycles (Plachot *et al.*, 1988). However, Bongso *et al.* (1988) found no significant difference between superovulatory drugs on the incidence of

chromosomal aberrations in oocytes. In animal research, Maudlin and Frazer (1977) reported that the incidence of chromosomal abnormality in embryos increased with increasing doses of pregnant mare serum gonadotrophin (PMSG) in superovulated mice.

Our knowledge of the metabolism of human embryos is limited (Gott *et al.*, 1990) and thus the culture conditions provided in human IVF are considered suboptimal as shown by human embryo development studies carried out *in vitro* using many types of media (Muggleton-Harris *et al.*, 1990). Therefore the incidence of chromosomal abnormalities in a particular IVF programme may represent the overall effect of various steps involved in the technique and a comparison with other reports of data obtained may help in selecting the best methods.

To study the chromosome constitution of oocytes and embryos generated in our IVF programme at PIVET Medical Centre, oocytes, zygotes and cleaving embryos donated by patients who were undergoing various assisted reproductive procedures were analysed. Ethical approval for the study was given by our Institutional Ethics Committee, and both partners signed informed consent forms in advance.

Materials and methods

Sixty-five patients who were undergoing assisted reproductive procedures at PIVET Medical Centre gave written consent to donate their excess oocytes, normal embryos, polypronuclear embryos and unfertilized oocytes for cytogenetic analysis. These patients were given subcutaneous injections of GnRH analogue, leuprolide acetate (Lucrin, Abbott, Australia), 1 mg (20 IU) daily, either from day 21 of the previous menstrual cycle (pituitary down-regulation) or from day 1 of the treatment cycle ('flare' effect) until ovulation was triggered with 10 000 IU of human chorionic gonadotrophin (HCG). In both instances human menopausal gonadotrophin (HMG, Pergonal, Serono) was given from day 3 of the treatment cycle with an increase in the dosage depending on the oestradiol rise. Oocytes were aspirated transvaginally with ultrasound guidance 35 h after the HCG trigger.

Oocytes and embryos were cultured in human tubal fluid (HTF) medium supplemented with 10% heat-inactivated patient's serum. Insemination was carried out 4–6 h after oocyte recovery with 50 000–100 000 spermatozoa/ml; the semen quality varied from severe oligozoospermia to normozoospermia. The presence of two pronuclei (normal fertilization) or three or more pronuclei (polypronuclear fertilization) 18–20 h after insemination confirmed that fertilization had occurred. All oocytes and embryos were graded on respective 1–4 point scales (Yovich and Lower, 1991) and the two or three of best quality were transferred to the patient.

Chromosome analysis of donated preovulatory oocytes was carried out within 6–24 h of collection. Most oocytes which failed to fertilize were analysed within 48–72 h of collection but some were analysed within 77–89 h due to incubation for variable lengths of time after addition of colcemid at 72 h. The excess donated embryos were prepared for chromosome analysis on the same day, or after culture for 1–3 days.

Some unfertilized oocytes and all the embryos were incubated with 0.1 μg/ml colcemid (Gibco) for 4–16 h depending on the stage of embryo development, prior to fixation for chromosome analysis. Cumulus cells were removed from the preovulatory oocytes using 0.1% hyaluronidase (H 3060, Sigma Chemical Co.) and all oocytes and embryos were prepared for chromosome analysis using the method described by Wramsby and Liedholm (1984). In brief, the oocytes and embryos were exposed to a hypotonic solution of 1% sodium citrate for 10 min. Then after a brief exposure to fixative A (5 parts H_2O:1 part acetic acid:4 parts ethanol) they were fixed on to glass slides by the addition of a few drops of fixative B (3 parts ethanol:1 part acetic acid). The slides were dried on a warming plate, stained with Giemsa and examined under an oil immersion objective lens.

The incidence of abnormal chromosomal complements and premature chromosome condensation (PCC) in unfertilized oocytes was correlated to the various patient criteria including the ovarian stimulation regimen, number of HMG ampoules used, peak oestradiol, age of patient and the number of previous attempts. The differences between patient groups were tested for significance using chi-square analysis.

Results

A total of 362 oocytes and embryos were analysed. Of the 193 unfertilized oocytes analysed (Table I), 103 (53.4%) gave metaphase plates. Of the metaphase plates, some were analysed only at the ploidy level and others were subjected to detailed cytogenetic analysis. Overall analysis of the metaphases indicated 78 (75.7%) as haploid and 25.2% with some form of chromosomal aberration, the most common being diploidy (19/26, 73.1%). A high incidence of PCC (17/103, 16.5%) was observed among unfertilized oocytes and the unfertilized oocytes exposed to colcemid showed a significantly higher incidence of PCC (14/62, 22.6%) than those untreated with colcemid (3/41, 7.3%, $P < 0.05$). In the colcemid-treated group, PCC was seen in 4 out of 14 oocytes fixed at 6–17 h after colcemid treatment of 72-h-old oocytes. A correlation between the incidence of chromosomal abnormality and PCC among unfertilized oocytes and patient criteria such as the ovarian stimulation regiment, number of HMG ampoules used, peak oestradiol, age of patient and the number of previous attempts was done. The findings are given in Table II and none of the criteria tested showed any significant effect on chromosomal abnormality in unfertilized oocytes. However, GnRH analogue in a 'flare' protocol was related to a significantly higher incidence of PCC (40%) than GnRH in a 'down-regulation' regimen (12.5%).

Similarly, a correlation test was performed between the incidence of chromosomal anomalies obtained for unfertilized oocytes and the day of HCG trigger, the corresponding oestradiol levels and the HMG dosage of 33 patients who showed some chromosomal aberration. High peak oestradiol levels were observed for patients who received HCG on day 10–12 (mean oestradiol of 12 000–15 000 pmol/l) and low oestradiol levels for HCG day >13 (mean oestradiol of 9000 pmol/l). The mean number of HMG ampoules used increased from day 11 (31 ampoules) to day >14 (72 ampoules). No significant difference was noted in the incidence of chromosomal abnormalities in relation to any of the HCG trigger days.

The results obtained for preovulatory oocytes are given in Table III. The incidence of chromosomal abnormality among

Table I. Chromosome analysis of unfertilized human oocytes

No. patients	No. oocytes analysed	No. with metaphases	Chromosome analysis						
			Haploid (n)	Fragmented Haploid (n)	Hypohaploid ($<n$)	Hyperhaploid ($>n$)	Diploid ($2n$)	Polyploid ($>2n$)	PCC
38	193	103 53.4%	77 75.7%	1	2	2	19	2	17[a]

[a]14/62 = 22.6% PCC (premature chromosome condensation) observed after incubation with colcemid; 3/41 = 7.3% PCC observed with no colcemid ($P < 0.05$).
Incidence of chromosome abnormality = 26/103 (25.2%).

Table II. Incidence of chromosome abnormalities and premature chromosome condensation (PCC) in unfertilized oocytes in relation to patient criteria

Unfertilized oocytes	Stimulation regimen		No. of HMG ampoules					Peak oestradiol (pmol/l)		Age of patient (years)		No. of previous stimulation attempts	
	GnRH flare	GnRH down-reg.	20–30	31–40	41–50	51–60	>61	5000–10 000	>10 000	<30	>30	Nil	One or more
No. with countable metaphases	15	88	23	23	16	30	11	38	64	40	63	19	84
No. with abnormal chromosomes (%)	4 (26.7)	22 (25.0)	4 (17.4)	9 (39.1)	4 (25.0)	6 (20.0)	3 (27.3)	11 (28.9)	15 (23.1)	9 (22.5)	17 (27.0)	2 (10.5)	24 (28.6)
No. with PCC (%)	6[a] (40.0)	11[b] (12.5)	1 (4.3)	5 (21.7)	6 (37.5)	4 (13.3)	1 (9.1)	6 (15.8)	11 (16.9)	5 (12.5)	12 (19.0)	3 (15.8)	14 (16.7)

[a] versus [b] = $P < 0.05$.
GnRH = gonadotrophin-releasing hormone; HMG = human menopausal gonadotrophin.

preovulatory oocytes was 16.7% (2/12) with the main chromosomal aberration being diploidy.

The embryos arising from two-pronuclear oocytes (normally fertilized) were graded for quality over a four-point scale and the incidence of chromosome abnormality was studied for good quality (>2/4) and poor quality (<1.5/4) embryos. Of 85 embryos, 26 had metaphases and 22 were suitable for analysis (see Table IV). Four of the 16 good quality embryos (23.5%) showed either haploid/diploid mosaicism, polyploidy or polyploid/diploid mosaicism. Of the poor quality or degenerated embryos, five out of six (83.3%) showed abnormal metaphases.

During the period when this study was undertaken 193 of the 1252 oocytes were confirmed polypronuclear at the time of pronuclear check, giving a rate of 7.3% polypronuclear fertilization. The description of the polypronuclear embryos used and their chromosome analysis are given in Table V. Less than half of the embryos showed metaphase plates (29/66, 43.9%) and only 22 were readable (i.e. 33.3% of the total). The majority of embryos showed polyploidy at both 1-cell and cleavage stages. Three of the readable 1-cell embryos showed PCC (3/10, 30%), 2 of these in haploid and 1 in a diploid spread of chromosomes. The total number of 1-cell and cleaved polypronuclear embryos with chromosome abnormality was 17/22, 77.3%. A small proportion of the cleaving embryos (4/17, 19%) gave only diploid metaphases.

Table III. Chromosome analysis of preovulatory oocytes

No. patients	No. oocytes analysed	No. with metaphases	Chromosome analysis	
			Haploid (n)	Diploid ($2n$)
4	18	12 66.7%	10 83.3%	2 16.7%

Incidence of chromosome abnormality = 2/12 (16.7%).

Discussion

The incidence of chromosomal abnormality among unfertilized oocytes in the present study (25.2%) is lower than the 47.7% described by Ma *et al.* (1989) but similar to the other reported figures, i.e. 22.2% (Veiga *et al.*, 1987), 23% (de Jong *et al.*, 1985), 11% (Spielmann *et al.*, 1985). A recent survey carried out by Pellestor (1991) on 1500 pooled oocyte chromosome complements indicated that the overall incidence of chromosomal abnormality in mature oocytes is 24.0%. The preovulatory oocytes which were not inseminated gave a chromosome anomaly rate of 16.7% and the oocytes which failed to fertilize *in vitro*, 25.2%. This rate of chromosomal abnormality is much lower than that described by Wrambsy *et al.* (1987) for preovulatory oocytes (47%). The incidence of chromosomal abnormality among embryos generated from normal fertilization (23.5%) is similar to that described elsewhere (Angell *et al.*, 1983; Michelmann and Mettler, 1985; Rudak *et al.*, 1985; Plachot *et al.*, 1988; Wimmers and Merwe, 1988). A high incidence of structural aberrations causing a high incidence of overall chromosomal anomalies in human embryos (40%) was reported by Papadopoulos *et al.* (1989). It is clear that there is a certain amount of variability in the incidence of chromosomal abnormality among human oocytes and embryos among different studies. Among the factors that may influence this variability are the fixation techniques for the preparation of chromosome spreads

Table IV. Chromosome analysis of cleaving embryos arising from normally fertilized oocytes

Quality of embryo	Grade of embryo[a]	Age of embryo[b]	Stage of embryo	Chromosome analysis									
				No. interphase nuclei	Total no. metaphases	Metaphases not countable	Metaphases countable						
							1	2	3	4	5	6	7	8
Good	3	27	4-cell		2		2*n*	46						
	3	48	4-cell	6	1	1								
	3	94	morula		2		2*n*	2*n*						
	3	94	morula		3		2*n*	2*n*	2*n*					
	3	94	morula		2	2								
	3	94	morula		1		cb*							
	2.5	74	6-cell	12	1		4*n*							
	2.5	74	8-cell	7	1		2*n*							
	2.5	74	8-cell	8	1		2*n*							
	2.5	92	morula	15	3		*n*	*n*	2*n*					
	2.5	92	morula	14	1		2*n*							
	2.5	115	morula	29	1	1								
	2.5	94	morula		2		2*n*	2*n*						
	2.5	75	blastocyst	89	3		2*n*	2*n*	2*n*					
	2	94	16-cell		4		46	46	46	46				
	2	94	16-cell		6		2*n*	2*n*	2*n*	2*n*	2*n*	2*n*		
	2	75	morula	23	1		2*n*							
	2	115	morula	6	8		46	2*n*	2*n*	2*n*	2*n*	2*n*	2*n*	2*n*
	2	115	morula		7	1	2*n*	2*n*	2*n*	2*n*	2*n*	>4*n*		
	2	115	morula	10	4	4								
Poor	1.5	51	4-cell	1	1		2*n*							
	1	50	3-cell		2		16	46						
	1	48	1-cell		1		40							
	1	75	deg. emb	8	1		>4*n*							
	1	75	deg. emb	28	2		3*n*	3*n*						
	0.5	75	2-cell		1		15							

[a]Grade of embryo: embryo quality graded on a 1–4 point scale.
[b]Age of embryos in hours from insemination to addition of colcemid.
*cb, chromosome break.

causing artefactual loss of chromosomes, the superovulation regimens, timing of the HCG trigger and oocyte recovery, the response of the patient to superovulatory drugs, culture conditions or sperm quality. In the present study, a large number of the metaphase spreads obtained using the gradual fixation technique described by Wramsby and Liedholm (1984) were difficult to analyse in detail, as in the case with Tarkowski's (1966) air drying method used by other investigators. This difficulty was due to the poor spreading of chromosomes and the condensed nature of chromatids in oocytes, as described by Pellestor (1991). Thus, in the present study most of the metaphases were analysed at the ploidy level except for those where the detailed chromosome numbers are given in the tables. Due to these limitations, the estimated frequency of chromosomal aberrations in oocytes and embryos may be lower than the actual incidence.

In unfertilized oocytes, a high proportion showed diploidy. The diploidy may be the result of unreleased polar body chromosomes or oocytes arrested at syngamy after sperm entry. There is evidence to show that gonadotrophins, particularly PMSG, have a tendency to increase the incidence of unreduced oocytes (Takagi and Sasaki, 1976) and to interfere with the cortical reaction giving rise to frequent dispermy (Fujimoto *et al.*, 1974).

The overall incidence of PCC (16.5%) among unfertilized oocytes in the present study is similar to that found by Ma *et al.* (1989). However, when the unfertilized oocytes that were not exposed to colcemid are considered, the incidence of 7.3% PCC among these oocytes is similar to that observed by Schmiady *et al.* (1986) in their first report on the incidence of PCC in developmentally arrested oocytes (7.4%). The explanation given by Schmiady *et al.* (1986) for the occurrence of this chromosomal abnormality is that oocytes are possibly arrested at metaphase II after sperm penetration and the continuing presence of cytoplasmic chromosome condensing factors causing the sperm nucleus to undergo chromosome condensation prematurely. The increased incidence of PCC in unfertilized oocytes when analysed after exposure to colcemid may represent either the true incidence of the abnormality in oocytes or an artefact caused by the fragmentation of chromatin due to degenerative changes in oocytes. As shown by Oritz *et al.* (1982), oocytes usually remain morphologically normal until at least 72 h after ovulation. In the present study, six unfertilized oocytes were analysed beyond this time limit in the colcemid treated group and four of them showed PCC. Thus, further investigation is required to study the effect of colcemid on a larger group of unfertilized oocytes.

Analysis of abortus material from women who had been induced to ovulate showed an increased incidence of chromosomal abnormality (83%) when compared to untreated women (60%) after in-vitro fertilization (Boué and Boué, 1973). However, a European survey carried out on spontaneous abortions after IVF (Plachot, 1989) showed no significant increase in chromosomal abnormality (62%) over natural conceptions (60%), a finding matched by studies at PIVET applying chorionic villus sampling to pregnancies diagnosed as failing (Yovich and Lower, 1991). Following analysis of oocytes recovered for IVF,

Table V. Chromosome analysis of one-cell and cleaving embryos arising from polypronuclear oocytes

Patient no.	Embryo no. (n = 29)	Age of embryo[a]	Stage of embryo	Chromosome analysis								
				No. of interphase nuclei	Total no. metaphases	Metaphases not countable	Metaphases countable					
							1	2	3	4	5	PCC
1	1	27	1-cell		1	1						
2	1	27	1-cell		1							$n+$
	2	27	1-cell		1							$n+$
	3	27	1-cell		1		$2n$					
3	1	27	1-cell		1		n					
4	1	26	1-cell		1		$3n$					
5	1	50	1-cell		1							$2n+$
	2	50	1-cell		1		$>4n$					
	3	50	1-cell		1		$>4n$					
	4	50	1-cell		1		$>4n$					
	5	50	1-cell		1	1						
6	1	27	1-cell		1		$4n$					
7	1	92	8-cell		5		n	$2n$	$2n$	$2n$	$2n$	
8	1	68	morula		1		$2n$					
9	1	51	8-cell		1		100+					
10	1	51	frag.		1		69					
	2	51	frag.		1	1						
11	1	51	8-cell	7	1		36					
12	1	51	8-cell	13	1	1						
13	1	27	3-cell		1		66					
14	1	51	8-cell	7	1	1						
15	1	72	8-cell	7	1	1						
16	1	77	morula	19	1		$2n$					
17	1	67	8-cell	9	1		$2n$					
18	1	50	3-cell		1		$>4n$					
19	1	50	3-cell	1	2		$2n$	$2n$				
	2	50	2-cell		2		$2n$	$>4n$				
20	1	77	8-cell	11	1	1						
21	1	72	4-cell	3	1		$3n$					

[a]Age of embryo in hours from insemination to addition of colcemid.
$n+$ or $2n+$ = hypoploid or diploid metaphase plus PCC.

some authors have suggested that superovulatory treatment might increase the incidence of chromosomally abnormal oocytes (Wramsby *et al.*, 1987). On the contrary, others have shown no significant influence of different stimulatory regimens on the chromosomal constitution of oocytes (Bongso *et al.*, 1988. Plachot *et al.*, 1988). In animal studies, there is evidence to show that gonadotrophins, especially PMSG, increase the incidence of chromosomal anomalies in embryos fertilized *in vitro* (Maudlin and Fraser, 1977). The differences observed in the effect of superovulatory drugs may be related to the patient group involved in each study. For example, at PIVET, once a patient has shown a poor response to the 'GnRH down-regulation' regimen, she is managed on the 'GnRH flare' regimen in the subsequent IVF attempts. Therefore it is legitimate to classify the patients who were placed on the 'GnRH flare' regimen in this study as difficult or poor responders. This latter group showed a significant increase in the incidence of PCC when compared to the 'GnRH down-regulation' group, thus indicating a nuclear defect causing the oocytes to arrest at metaphase II after sperm entry. A larger study group is required to confirm these findings. Furthermore, a controlled cytogenetic study involving oocytes and preimplantation embryos, from both spontaneous cycles and hormonally induced cycles, may indicate the effect of superovulatory drugs on the incidence of chromosomal abnormalities at these early embryonic stages.

When various patient criteria were tested for correlation with the incidence of chromosomal abnormalities, none of the criteria tested, such as HMG dosage, peak oestradiol level, age of patient and number of previous attempts, affected the incidence of chromosomal abnormalities. This finding is similar to that reported by others (Plachot *et al.*, 1988). The dosage of HMG used for superovulation could be determined by the patient group, i.e. whether the patient is a good or poor responder. Thus, when the distribution of HMG dosage is calculated according to the length of the follicular phase indicated by the day of the HCG trigger, high HMG dosages and low peak oestradiol levels were observed when the follicular phase was >14 days (data not shown). However, no significant correlation was seen between the incidence of chromosomal abnormality and whether subjects were good or poor responders. Thus, further studies are needed to assess the factors which contribute towards increasing chromosomal anomalies in oocytes.

The chromosome complement of embryos originating from normally fertilized oocytes depended largely on the embryo quality, where 83% of the fragmenting embryos (grading <1.5 out of a maximum of 4 points) gave grossly abnormal chromosome complements. The incidence of chromosomal abnormalities among good and poor quality embryos is similar to that described previously (Plachot *et al.*, 1987, 1988). The cause of fragmentation in embryos could be inherent

chromosomal defects in the embryos themselves or exposure to adverse environmental conditions during in-vitro culture. In our experience, IVF pregnancy rates tend to fluctuate mainly as a reflection of the quality of water used in the preparation of culture media (Yovich *et al.*, 1988). Usually a low pregnancy rate during a particular period is associated with a high rate of pregnancy loss. Furthermore, due to the limited knowledge of human embryo metabolism (Gott *et al.*, 1990) and thus of the culture requirements for human embryos, the culture conditions provided in IVF are suboptimal, as indicated in the embryo developmental studies carried out by Muggleton-Harris *et al.* (1990).

During the period when this study was carried out, the incidence of polypronuclear embryos was 7.3%. This rate is comparable to the incidence reported by Trounson *et al.* (1982) but higher than for some other IVF programmes (Michelmann *et al.*, 1986; Rudak *et al.*, 1984). These differences may relate to the completeness of oocyte recovery rather than any adverse laboratory factor. For example, oocyte recovery at PIVET utilizes a double-lumen flushing needle (PIVET-Cook; Cook Aust Pty Ltd) by the transvaginal approach, with >90% oocytes recovered from all follicles of ≥10 mm diameter (Yovich and Grudzinskas, 1990). This may cause the retrieval of a higher proportion of oocytes susceptible to polyspermia, although this comment is speculative at this stage.

Cytogenetic analysis of these polypronuclear embryos gave gross abnormal chromosome complements for the majority of the embryos and normal diploid numbers for a few. Similar observations have been made in previous studies (Trounson, 1982; Rudak *et al.*, 1984; Angell *et al.*, 1986; Michelmann *et al.*, 1986) and the possible modes of division of multipronuclear embryos have been discussed by Angell *et al.* (1986) in the process of generating blastomeres with various numbers of chromosomes. The explanation given for the origin of diploid embryos is the exclusion of excess chromosome complements at the first cleavage division, thus restoring $2n$ or 46 chromosomes. Triploid embryos possibly result from division of all chromosomes, so producing daughter cells with a full triploid complement. The embryos with polyploid chromosome complements may not continue to divide, as the cytogenetic analysis of abortus material indicates that triploidy is a very rare occurrence (Plachot, 1989). When polypronuclear embryos give rise to cells bearing a diploid chromosome complement, the developmental potential of these embryos depends on the percentage of normal diploid cells. In the present study, the two embryos that were analysed at the morula stage displayed a diploid set of chromosomes (Table V), suggesting their normal developmental potential over the other embryos with polyploidy which had arrested at early stages.

To conclude, the incidence of chromosomal abnormality in oocytes (unfertilized and preovulatory) and embryos (normal and polypronuclear) is within the ranges described by other investigators for these early embryonic stages generated under different IVF conditions. Routine cytogenetic analysis of oocytes and embryos that are not destined for transfer to the patient will provide valuable information for assessing the various procedures involved in IVF and determining the prognosis for differing sub-categories of patients. Ultimately, further attempts should be made to study the optimal culture requirements for the generation of healthy embryos with a low incidence of cytogenetic abnormalities, in order to improve the pregnancy outcome in human IVF.

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