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Aneuploidy screening in coelomic samples using fluorescence *in situ* hybridisation (FISH)

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Objectives Coelocentesis is the earliest invasive prenatal diagnostic procedure that has recently been used in ongoing pregnancies to identify single gene defects. Aneuploidy screening has not yet been performed in ongoing pregnancies following coelocentesis, but experimental studies have demonstrated the ability of determining the copy number of chromosomes 13, 18, 21, X and Y in uncultured coelomic samples, by FISH or PCR. The aim of this study was to extend previous studies and investigate the feasibility of analysing uncultured coelomic fluid samples for 11 chromosomes using fluorescence *in situ* hybridisation (FISH).

Methods Coelocentesis was performed in 12 singleton pregnancies at 6 to 9 weeks of gestation immediately before surgical termination of pregnancy. Fluorescence probes for chromosomes 3,7,9,13,16,17,18,21,22, X and Y were applied on uncultured coelomic-fluid samples and placental tissue. In cases where coelomic cells were not of a sufficient amount, chromosomes X and Y were analysed in a second layer of hybridisation.

Results Successful analysis by FISH was possible in all cases and the results from the coelomic fluid were concordant with those from the analysis of placental tissue and obtained within a few hours of receiving the samples. Problems associated with limited cell numbers were overcome by the application of a second layer of FISH. This sequential approach has also enabled accurate identification of maternal-cell contamination in male samples.

Conclusion Analysis of 11 chromosomes using FISH in coelomic fluid samples is feasible and it has the potential to be applied for rapid aneuploidy screening, should coelocentesis be used clinically as an early, invasive prenatal diagnostic tool. Copyright © 2005 John Wiley & Sons, Ltd.

KEY WORDS: coelomic fluid; placenta; interphase FISH; aneuploidy

INTRODUCTION

Coelocentesis involves the ultrasound-guided transvaginal insertion of a needle into the extra-amniotic cavity and aspiration of coelomic fluid at 6 to 10 weeks of gestation (Jurkovic *et al*., 1993). The technique has been successfully applied in the prenatal diagnosis of single gene defects, such as ß-thalassaemia and sickle cell disease, by DNA analysis (Makrydimas *et al*., 2004). However, coelocentesis has not been used for fetal karyotyping in clinical studies because of the difficulty in culturing coelomic samples to obtain metaphases for conventional cytogenetic analysis (Jurkovic *et al*., 1993; Cruger *et al*., 1996). Nevertheless, experimental studies have demonstrated the ability of determining the number of chromosomes 13, 18, 21, X and Y in uncultured coelomic samples, by molecular cytogenetic approaches, including fluorescence *in situ* hybridisation (FISH) (Pandya *et al*., 1995; Cruger *et al*., 1997) and polymerase chain reaction (PCR) (Findlay *et al*., 1996; Jauniaux *et al*., 2003).

The aim of this study was to investigate the feasibility of analysing uncultured coelomic samples for 11 chromosomes using the FISH approach.

MATERIALS AND METHODS

FISH was carried out in 12 coelomic fluid- and placental tissue samples collected from women undergoing termination of pregnancy for psychological reasons at the University Hospital of Ioannina. The study was approved by the Ethics Committee of the hospital, and in all cases the women gave informed written consent. Coelocentesis was performed under general anaesthesia immediately before pregnancy termination. The external genitalia and the vagina were cleansed with an antiseptic solution, transvaginal sonography (5 MHz transducer, Toshiba SSA-220A, Tokyo, Japan) was performed and the embryo, amniotic membrane, coelomic space and yolk sac were identified. A 20-G needle was then introduced transvaginally into the coelomic cavity, through a guide attached to the transducer and fluid was aspirated. The first sample of 0.2 mL was discarded to avoid contamination with maternal tissue and a new syringe was then used to aspirate 1 to 4 mL. A sample of placental tissue was obtained after the surgical evacuation of the

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products of conception and placed in a tube containing sterile culture medium (RPMI-1640, Cambrex Bio Science, Wokingham, UK). The samples were sent to the UK for analysis and the interval between sampling and arrival in the laboratory was 2–3 days.

Preparation of samples

Coelomic fluid samples were centrifuged at 1500 rpm for 5 min and the pellet was either fixed in 3 : 1 methanol/acetic acid and dropped on poly-L-lysine slides (BDH, Québec, Canada) or after being resuspended in 10 μL phosphate-buffered saline (PBS- Sigma-Aldrich, Gillingham, Dorset UK), the cells were spread on poly-L-Lysine slides (BDH, Québec, Canada) using 0.01 N HCL (BDH, Québec, Canada), 0.1% Tween-20 (Sigma–Aldrich, Gillingham, Dorset UK). The slides were left to air-dry, washed in PBS (Sigma–Aldrich, Gillingham, Dorset UK), dehydrated through 70%, 90% and 100% ethanol series (BDH, Québec, Canada) and left to air-dry.

Placental tissue was washed in Ethanol to get rid of maternal cells and the villi were rubbed on poly-L-Lysine slides (BDH, Québec, Canada) (Moore *et al*., 2000). The slides were left to air-dry, fixed twice in 3 : 1 methanol/acetic Acid, dehydrated through 70%, 90% and 100% ethanol series and left to air-dry.

The hybridisation efficiencies of the three chromosome-specific probe sets used were tested using control interphase lymphocyte slides. Blood from a normal male (46,XY) was collected and lymphocyte extraction and fixation was carried out according to standard protocols. In brief, 2.5 mL of blood was diluted 1 : 1 in PBS (Sigma Sigma-Aldrich, Gillingham, Dorset UK) and carefully poured on top of 5-mL Ficoll–Plaque (Pharmacia, Sweden) in a 15-mL tube, taking care to avoid the two liquids getting mixed. A 20-min centrifugation at 1600 rpm followed, to separate the cell-free plasma (upper layer), lymphocytes (middle layer), Ficoll-Plaque solution (bottom layer) and red blood cells and debris (pellet). After centrifugation, the narrow lymphocyte band, formed at about the cell suspension/Ficoll–Plaque interphase was aspirated and resuspended in 10 mL PBS (Sigma Sigma–Aldrich, Gillingham, Dorset UK). Centrifugation at 1200 rpm for 10 min followed, the supernatant was discarded, the pellet was resuspended in 10 mL PBS (Sigma Sigma–Aldrich, Gillingham, Dorset UK) and the centrifugation step was repeated. Finally, the supernatant was discarded and the pellet was fixed in 3 : 1 methanol : acetic acid by dropwise addition of the fixative (10 mL). Centrifugation at 1000 rpm for 10 min followed, the supernatant was discarded and the pellet was fixed again in 3 : 1 methanol : acetic acid. The fixation step was repeated again and the tubes were centrifuged at 1000 rpm for 10 min (The pellet looked white and the supernatant was clear). Finally, 20 μL of fixed lymphocytes was dropped on slides and the remaining lymphocyte suspension was stored at −20 °C for later use. Slides of these lymphocyte preparations were hybridised using the same protocol as for the coelomic and placenta samples. Three experiments were performed and a total of six slides (two slides per experiment) were processed for FISH and analysed.

Fluorescence *in situ* hybridisation (FISH)

Fluorescence *in situ* hybridisation was performed according to Harper *et al*. (1994) with modifications. The slides were incubated in 100 μg/mL pepsin (Sigma-Aldrich, Gillingham, Dorset UK) in 0.01 N HCL (BDH, Québec, Canada) at 37 °C for 20 min, washed in distilled water and PBS and incubated in 1% paraformaldehyde in PBS at 4 °C for 10 min. Following two further washes in PBS and distilled water, the slides were dehydrated though 70%, 90% and 100% ethanol series, and air-dried. We used three sets of probes, Multivysion PB (CEP18, Spectrum Blue; CEP22, Spectrum Gold; CEP16, Spectrum Aqua; LSI13, Spectrum Red; LSI21, Spectrum Green), Aneuvysion (CEPX, Spectrum Green; CEPY, Spectrum Orange; CEP18, Spectrum Aqua) and Urovysion (CEP3, Spectrum orange; CEP7, Spectrum Green; CEP9, Spectrum Gold; CEP17, Spectrum Aqua) (Abbott Molecular Diagnostics, Chicago, Illinois). The probe sets were applied under a coverslip, sealed with rubber solution and placed in the Hybrite (Abbott Molecular Diagnostics, Chicago, Illinois) for codenaturation and hybridisation. The codenaturation and hybridisation conditions for the Multivysion and Urovysion probe sets were 75 °C for 5 min and 37 °C for 5 h to overnight respectively, while for the Aneuvysion probe set the conditions were 75 °C for 5 min and 37 °C for 2 h.

Following hybridisation, the slides were washed in 0.4x Standard Saline Citrate (SSC; Abbott Molecular Diagnostics, Chicago, Illinois)/0.3% Tween-20 (Sigma-Aldrich, Gillingham, Dorset UK) at 73 °C for 3 min and briefly in 2 × SSC/0.1% Tween-20 (Sigma–Aldrich, Gillingham, Dorset UK). They were then air-dried, mounted in Vectashield antifade medium (Vector Laboratories, CA, USA) containing 1 ng/mL DAPI (4,6,-diamidino-2-phenylidole) (Sigma) under a coverslip and sealed with nail varnish. (Samples hybridised with the Multivysion PB probe set were mounted in Vectashield antifade medium without DAPI). Nuclei were examined by fluorescence microscopy using the Olympus BX61 and images captured using the Cytovysion software (Applied Imaging, Newcastle, UK). The scoring criteria previously published by Hopman *et al*. (1988) were strictly adhered to. On the basis of these criteria, nuclei were classified as aneuploid if they contained one extra or missing signal for up to two chromosomes. Nuclei possessing a random distribution of signals for three or more chromosomes were classified as chaotic (Delhanty *et al*., 1997). Nuclei containing exact multiples of the diploid number of signals were classified as polyploid.

In cases where coelomic cells were not of a sufficient quantity to be fixed on three target areas, two target areas were prepared and hybridised with the Multivysion PB and Urovysion probe set respectively. Then, a sequential FISH approach was used to analyse chromosomes X and Y in a second layer of hybridisation. This sequential

FISH approach was also used in some placental samples and was initially optimised on control lymphocytes.

Sequential FISH involved washing the slides in 4 × SSC for 10 min at RT, following analysis of the first layer. The slides were dehydrated through 70%, 90% and 100% ethanol series and left to air-dry, the Aneuvysion probe was applied under a coverslip, sealed with rubber solution and the slides were placed in the Hybrite (Abbott Molecular Diagnostics, Chicago, Illinois) for codenaturation at 75 °C for 3 min and hybridisation at 37 °C overnight. Post-hybridisation washes and nuclei examination were performed as detailed above.

RESULTS

The Multivysion PB, Urovysion and Aneuvysion probe sets hybridised with high efficiencies on control interphase lymphocytes (Table 1). Table 2 shows the hybridisation efficiency of the Aneuvysion probe set, when applied as a second layer.

FISH was successfully performed with all three probes in both the coelomic fluid and placental samples (Table 3, Figure 1), and the results were obtained within the same day of receiving the samples in most cases. Where possible, a maximum of a hundred cells were counted for each of the probe set used. In samples with an insufficient number of cells present, the X,Y probe was analysed in a second layer of hybridisation by re probing the slide that contained the cells analysed with the Multivysion probe set (Figure 2).

None of the coelomic fluid samples examined had the same chromosome complement in all the nuclei analysed. Tetraploid cells and aneuploid cells were observed at low levels (<8%), which could reflect genuine mosaicism, due to cytokinetic failure, non-disjunction or chromosome loss, or simply represent technical artefacts. Samples with less than 10% abnormal cells were considered to be normal diploid.

In all cases there was concordance between the coelomic fluid and placental results, and based on the criterion mentioned above the samples were considered to be normal diploid, six being female and six male.

Table 1—Hybridisation efficiencies of the three chromosome-specific probe sets used on control interphase lymphocytes

Chromosome X	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	100			99	1	
Slide 2	98	2				
Slide 3	99	1		99.5	0.5	
Slide 4	100					
Slide 5	98	2		98.5	1.5	
Slide 6	99	1				
Mean ± SE				99.0 ± 0.3	1.0 ± 0.3	
Chromosome Y	**•**	**••**	**•••**	**Mean •**	**Mean ••**	**Mean •••**
Slide 1	100			99.5	0.5	
Slide 2	99	1				
Slide 3	98	2		98.5	1.5	
Slide 4	99	1				
Slide 5	99	1		99	1	
Slide 6	99	1				
Mean ± SE				99.0 ± 0.3	1 ± 0.3	
Chromosome 18	**•**	**••**	**•••**	**Mean •**	**Mean ••**	**Mean •••**
Slide 1		98	2	1.5	97.5	1
Slide 2	3	97				
Slide 3	2	96	2	1.5	96.5	2
Slide 4	1	97	2			
Slide 5	1	98	1	2	97	1
Slide 6	3	96	1			
Mean ± SE				1.7 ± 0.2	97.0 ± 0.3	1.3 ± 0.3
Chromosome 3	**•**	**••**	**•••**	**Mean •**	**Mean ••**	**Mean •••**
Slide 1	1	96	3	1	96.5	2.5
Slide 2	1	97	2			
Slide 3	3	95	2	1.5	96.5	2
Slide 4		98	2			
Slide 5	2	96	2	2.5	95	2.5
Slide 6	3	94	3			
Mean ± SE				1.7 ± 0.4	96.0 ± 0.5	2.3 ± 0.2

(*continued overleaf*)

Table 1—(*Continued*)

Chromosome 7	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	2	98		2	96.5	1.5
Slide 2	2	95	3			
Slide 3	1	98	1	1	97	2
Slide 4	1	96	3			
Slide 5	2	97	1	2	96.5	1.5
Slide 6	2	96	2			
Mean 1–6 ± SE				1.7 ± 0.3	96.7 ± 0.2	1.7 ± 0.2
Chromosome 9	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	4	95	1	3	95.5	1.5
Slide 2	2	96	2			
Slide 3	1	97	2	1	98	1
Slide 4	1	99				
Slide 5	1	96	3	1	97	2
Slide 6	1	98	1			
Mean ± SE				1.7 ± 0.7	96.8 ± 0.7	1.5 ± 0.3
Chromosome 17	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1		98	2	1.5	96.5	2
Slide 2	3	95	2			
Slide 3	2	97	1	1.5	96.5	2
Slide 4	1	96	3			
Slide 5	2	96	2	2	97	1
Slide 6	2	98				
Mean ± SE				1.7 ± 0.2	96.7 ± 0.2	1.7 ± 0.3
Chromosome 13	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	3	96	1	2	97	1
Slide 2	1	98	1			
Slide 3	1	97	2	1.5	96.5	2
Slide 4	2	96	2			
Slide 5	1	97	2	2	96	2
Slide 6	3	95	2			
Mean ± SE				1.8 ± 0.2	96.5 ± 0.3	1.7 ± 0.3
Chromosome 16	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	1	98	1	1.5	97	1.5
Slide 2	2	96	2			
Slide 3	2	95	3	2.5	94.5	3
Slide 4	3	94	3			
Slide 5	1	96	3	1.5	96.5	2
Slide 6	2	97	1			
Mean ± SE				1.8 ± 0.3	96.0 ± 0.8	2.2 ± 0.4
Chromosome 21	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1	1	97	2	1.5	96.5	2
Slide 2	2	96	2			
Slide 3	1	96	3	2	95	3
Slide 4	3	94	3			
Slide 5	1	98	1	2	97	1
Slide 6	3	96	1			
Mean ± SE				1.8 ± 0.2	96.2 ± 0.6	2.0 ± 0.6
Chromosome 22	•	••	•••	Mean •	Mean ••	Mean •••
Slide 1		99	1	0.5	98	1.5
Slide 2	1	97	2			
Slide 3		98	2	1	97	2
Slide 4	2	96	2			
Slide 5	2	97	1	2.5	96.5	1
Slide 6	3	96	1			
Mean ± SE				1.3 ± 0.6	97.2 ± 0.4	1.5 ± 0.3

The percentage of normal diploid cells was 92 to 97% for the chromosomes identified by the multivysion probe set and 91 to 96% for the urovysion probe. In two male diploid samples there was a low level of maternal cell contamination (4% and 6% respectively).

Maternal cell contamination was more prominent in the placental tissue than in the coelomic cells and identification of the contamination easily occurred in male samples after hybridisation of the X,Y probe. However, the extent of maternal cell contamination in normal female samples was not possible to quantify. Maternal cell contamination as identified by the existence of two signals for chromosome X and no signals for chromosome Y ranged between 2% and 7%. Figures 2c and d show sequential FISH analysis of a placental cell from sample 5, documenting a normal diploid male chromosomal constitution, while another cell from the same sample (e–f) represents maternal cell contamination. Tetraploid, aneuploid and chaotic cells were also observed at low levels.

Table 2—Hybridisation efficiency of the Aneuvysion probe set as a 2nd layer

Chromosome X	•	••	No signals
Slide 1	98		2
Slide 2	98	1	1
Slide 3	99		1
Slide 4	97	1	2
Mean ± SE	98 ± 0.4	0.5 ± 0.3	1.5 ± 0.3
Chromosome Y	•	••	No signals
Slide 1	99		1
Slide 2	97		3
Slide 3	98		2
Slide 4	97	1	2
Mean ± SE	97.8 ± 0.5	0.2 ± 0.2	2 ± 0.4
Chromosome 18	•	••	•••
Slide 1	1	98	1
Slide 2	4	95	1
Slide 3	2	98	0
Slide 4	3	97	0
Mean ± SE	2.5 ± 0.6	97 ± 0.7	0.5 ± 0.3

In contrast to the coelomic cells that were intermixed during spinning and therefore were not spread in a controlled way, placental cells were spread and fixed on slides in a controlled manner, which allowed accurate estimation of missing and extra signals in adjacent cells, suggesting non-disjunction. We therefore provide evidence that non-disjunction was a genuine phenomenon in the placental tissue. We also suggest that cytokinetic failure leading to tetraploid cells was a real event in both coelomic and placental cells, as those were never observed in control interphase lymphocyte nuclei.

DISCUSSION

This study has demonstrated the feasibility of examining coelomic fluid for the copy number of 11 chromosomes, including 13, 16, 18, 21, 22, X and Y, which are the ones involved in the most common aneuploidies. Successful analysis by FISH was possible in all cases and the results from coelomic fluid were concordant with those from the analysis of placental tissue. In addition, analysis was successfully performed within a few hours of receiving the samples.

Low-level mosaicism, including aneuploid, tetraploid and chaotic cells, was observed in all the coelomic fluid and placental samples examined. Tetraploid and chaotic cells were, however, more frequently observed in placental samples. The former are thought to arise through failure in karyokinesis and cytokinesis following DNA replication, are present at all stages of preimplantation development and are even considered to be part of normal development (Hardy *et al*., 1993). Suboptimal culture conditions may increase the incidence of cytokinetic failure, and although the present samples were uncultured, suboptimal conditions during transfer of the samples to the cytogenetic laboratory may have contributed to the formation of polyploid cells. Chaotic chromosomal complements may have been due to multipolar spindles, which can form when cells contain extra centrosomes, following cytokinetic failure (Chatzimeletiou *et al*., 2005), or represent technical artefacts. Aneuploid cells included monosomic and trisomic cells for one or more chromosomes and those were observed at similar levels in both coelomic and placental samples.

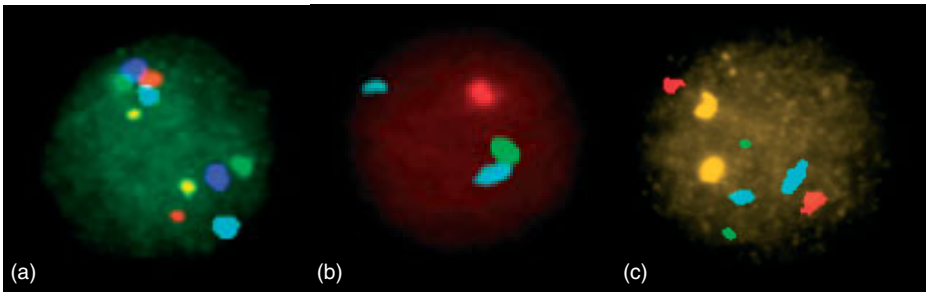

Figure 1—Photomicrographs showing fluorescence *in situ* hybridisation on coelomic fluid samples. Hybridisation of: (a) Multivysion PB probe set (chromosomes 13 (red), 16 (aqua), 18 (blue), 21 (green) and 22 (gold)). (b) Aneuvysion probe set (chromosomes X (green), Y (red) and 18 (aqua)) (c) Urovysion probe set (chromosomes 3 (red), 7 (green), 9 (gold), 17 (aqua) in coelomic cells from sample 9. Note that there are two signals for each chromosome, except for one red signal for chromosome Y and one green signal for chromosome X (b), denoting a diploid male chromosomal constitution

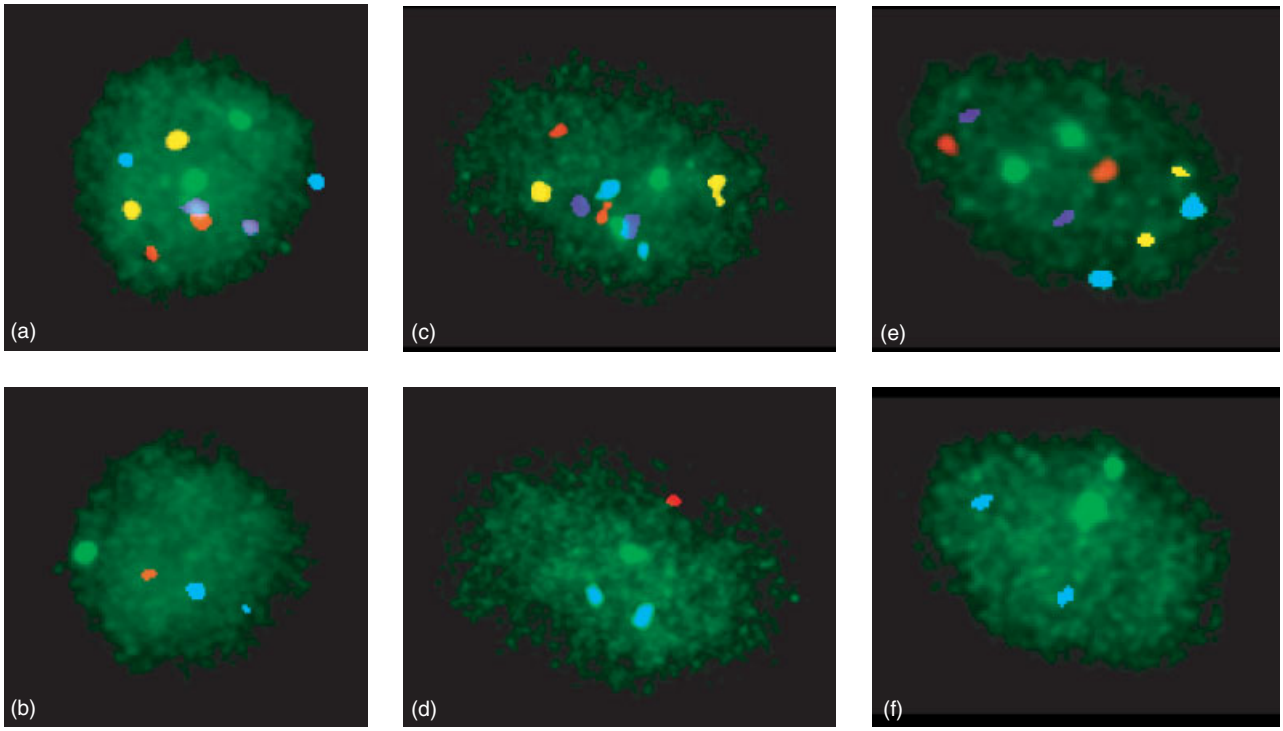

Figure 2—Photomicrographs illustrating sequential fluorescence *in situ* hybridisation. Hybridisation of the Multivysion PB probe set on a coelomic cell (a) from sample 5 demonstrating two signals for all the chromosomes. On account of the limited number of cells in the sample, chromosomes X and Y were analysed as a second layer (b). Note that there is one red signal for chromosome Y, one green signal for chromosome X, and two aqua signals for chromosome 18, confirming a normal male complement. Also note that the blue signals in (a) and aqua signals in (b), that are specific for chromosome 18, are in the same position in both layers. Sequential FISH analysis of a placental nucleus from the same sample hybridised with the Multivysion PB and the Aneuvysion probe set is shown in (c) and (d) respectively. Note the two signals for all chromosomes tested in (c) and the normal male chromosomal constitution in (d). In (e) and (f) sequential FISH analysis of a nucleus from the same sample is demonstrated showing a diploid complement when hybridised with the Multivysion PB probe set (e), but re probing of the same cell with the Aneuvysion probe set revealed a normal female complement (f), suggesting maternal cell contamination

These could be the result of non-disjunction, or technical artefacts. Monosomic cells might also have arisen through chromosome loss during anaphase (anaphase lagging) (Chatzimeletiou *et al*., 2005). Although maternal cell contamination was more prominent in the placental tissue than in the coelomic cells, identification of the contamination easily occurred in male samples after hybridisation of the X,Y probe (Figure 2).

The feasibility of performing sequential FISH in 2 layers of hybridisation to overcome the problems associated with limited cell numbers, as demonstrated in this study, could also be particularly useful in distinguishing between genuine mosaicism and maternal cell contamination when a mixture of diploid and aneuploid cells are demonstrated in the same sample in male pregnancies. Thus, in maternal cell contamination re probing with the X and Y probe would demonstrate that the diploid cells are female and the aneuploid ones are male. The fact that interphase molecular cytogenetics can accurately identify numerical chromosomal aberrations opens up the possibility of analysing prenatal samples without the need for extensive culture protocols. It is now possible with commercially available probes to screen samples for all 23 pairs of chromosomes using FISH. This could be done by either hybridising more target areas on the slides with different probes or by re probing the existing slides. In our study two sequential hybridisation layers were successfully applied without compromising signal quality and probe hybridisation efficiency. Nevertheless, the efficiency of such approach could decrease if it became necessary to undertake re probing in several layers.

There are three main limitations to the introduction of coelocentesis for fetal karyotyping as an alternative to amniocentesis or chorionic villous sampling. Firstly, the number of studies that have examined the safety of the technique is small. These studies have reported that coelocentesis is not associated with significant feto-maternal haemorrhage or alteration in fetal heart rate (Makrydimas *et al*., 1997a, b). A study of women undergoing coelocentesis 1 to 3 weeks before elective termination found that the rate of fetal death during this interval was only about 1% higher than in matched controls that had not undergone coelocentesis (Makrydimas *et al*., 2002). In a total of 12 pregnancies that continued after coelocentesis, 11 resulted in the delivery at term of healthy and appropriately grown neonates and one in preterm delivery at 25 weeks due to cervical incompetence (Ross *et al*., 1997; Makrydimas *et al*., 2002, 2004). Secondly, conventional cytogenetic analysis is not a realistic approach because of the difficulty in culturing coelomic samples. Interphase cytogenetics by FISH, on the other hand, can provide diagnosis of the common aneuploidies but cannot detect the majority of

Table 3—The chromosomal constitution of the 12 coelomic fluid samples and placentae

		Multivysion PB probe set Chromosomes 13,16,18,21,22				Urovysion probe set Chromosomes 3,7,9,17				Aneuvysion Probe set Chromosomes X,Y,18				
Sample	Number	Total *n*	No. of diploid cells (%)	No. of aneuploid cells (%)	No. of polyploid & chaotic cells (%)	Total *n*	No. of diploid cells (%)	No. of aneuploid cells (%)	No. of polyploid & chaotic cells (%)	Total *n*	No. of XY cells (%)	No. of XX cells (%)	No. of aneuploid cells (%)	Chromosomal constitution
Coelomic	1	100	93 (93)	6	1	100	96 (96)	4	0	100	98 (98)		2	Diploid male
	2	100	92 (92)	8	0	100	94 (94)	6	0	100	97 (97)		3	Diploid male
	3	100	95 (95)	5	0	58	54 (93.1)	4	0	100		98 (98)	2	Diploid female
	4	100	96 (96)	4	0	70	67 (95.7)	3	0	100	92 (92)	4	4	Diploid male[a,b]
	5	100	94 (94)	4	2	100	93 (93)	7	0	100	91 (91)	6	3	Diploid male[a,b]
	6	100	95 (95)	4	1	100	94 (94)	3	3	100	96 (96)		4	Diploid male
	7	100	95 (95)	5	0	75	72 (96)	3	0	100		98 (98)	2	Diploid female
	8	100	93 (93)	5	2	54	52 (96.3)	2	0	100		96 (96)	4	Diploid female[b]
	9	100	94 (94)	6	0	100	91 (91)	9	0	100	97 (97)		3	Diploid male
	10	100	97 (97)	3	0	69	65 (94.2)	4	0	100		98 (98)	2	Diploid female
	11	100	96 (96)	4	0	100	95 (95)	5	0	100		95 (95)	5	Diploid female
	12	100	95 (95)	5	0	48	46 (95.8)	2	0	100		98 (98)	2	Diploid female
Placenta	1	100	85 (85)	7	8	100	88 (88)	8	4	100	92 (92)	5	3	Diploid male[a]
	2	100	88 (88)	6	6	100	90 (90)	8	2	100	96 (96)	2	2	Diploid male[a]
	3	100	89 (89)	7	4	100	92 (92)	5	3	100		97 (97)	3	Diploid female
	4	100	92 (92)	5	3	100	95 (95)	4	1	100	90 (90)	7	3	Diploid male[a,b]
	5	100	88 (88)	5	7	100	89 (89)	7	4	100	95 (95)	4	1	Diploid male[a,b]
	6	100	90 (90)	5	5	100	95 (95)	4	1	100	94 (94)	3	3	Diploid male[a]
	7	100	86 (86)	8	6	100	90 (90)	6	4	100		96 (96)	4	Diploid female
	8	100	90 (90)	6	4	100	94 (94)	4	2	100		97 (97)	3	Diploid female
	9	100	87 (87)	5	8	100	93 (93)	6	1	100	93 (93)	5	2	Diploid male[a]
	10	100	90 (90)	7	3	100	92 (92)	5	3	100		97 (97)	3	Diploid female
	11	100	92 (92)	5	3	100	89 (89)	9	2	100		96 (96)	4	Diploid female
	12	100	91 (91)	5	4	100	94 (94)	4	2	100		98 (98)	2	Diploid female

[a] Maternal contamination.
[b] XY analysed as a 2nd layer.

chromosomal rearrangements, some of which are clinically significant. This problem could potentially be overcome with the use of other molecular cytogenetic techniques, like comparative genomic hybridisation (CGH) or microarrays, which do not require cells in metaphase and can screen the whole genome for global gains and losses. Although CGH has been successfully applied on chorionic villus samples and amniocytes (Lapierre *et al*., 2000; Lestou *et al*., 2000; Tabet *et al*., 2001) it is currently expensive and labour intensive. Thirdly, screening for chromosomal defects has shifted from the traditional approach of maternal age to fetal nuchal translucency and maternal serum free ß-hCG and PAPP-A at 11 to 13^{+6} weeks or second-trimester maternal serum biochemistry. Consequently, the need for invasive testing for chromosomal defects at 6 to 10 weeks would require the identification of effective sonographic markers in the fetus and/or biochemical markers in the mother.

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