Human Reproduction Vol.17, No.2 pp. 446–451, 2002

Cytogenetic analysis of miscarriages from couples with recurrent miscarriage: a case–control study

M.D.Stephenson[1,3,4,5], K.A.Awartani[1] and W.P.Robinson[2,3]

[1]Department of Obstetrics and Gynaecology and [2]Department of Medical Genetics, University of British Columbia, [3]British Columbia's Research Institute for Children's & Women's Health and [4]British Columbia's Women's Hospital & Health Centre, Vancouver, BC, Canada

[5]To whom correspondence should be addressed at: Women's Health Centre, 4500 Oak Street, Vancouver, BC, Canada V6H 3N1. E-mail: mstephenson@cw.bc.ca

BACKGROUND: Reproductive loss carries immeasurable human costs as well as being costly to the health care system. The objectives of this study were to determine the frequency and distribution of cytogenetically abnormal miscarriages from couples with recurrent miscarriage and to compare the results with the general population. METHODS: A total of 420 specimens, including 29 pre-clinical, 237 embryonic and 154 fetal, were successfully karyotyped from 285 couples with recurrent miscarriage. The results were stratified according to maternal age and compared with controls. RESULTS: In all, 225 specimens (54%) were euploid. A total of 195 specimens (46%) were cytogenetically abnormal, of which 131 (66.5%) were trisomic, 37 (19%) were polyploid, 18 (9%) were monosomy X, eight (4%) were unbalanced translocations and one was a combination of trisomy 21 and monosomy X. The frequency of euploid miscarriages was significantly higher in women <36 years of age with recurrent miscarriage compared with controls. The distribution of cytogenetic abnormalities in the recurrent miscarriage group was not significantly different from controls, when stratified by maternal age. CONCLUSIONS: Women <36 years of age with recurrent miscarriage have a higher frequency of euploid miscarriage. When stratified for maternal age, there is no difference in the distribution of cytogenetically abnormal miscarriages in couples with recurrent miscarriage compared with controls.

Key words: aneuploid/chromosomes/cytogenetic analysis/recurrent miscarriage/spontaneous abortion

Introduction

Miscarriage, defined as spontaneous pregnancy loss of <20–28 weeks gestation, is a common clinical problem. It is estimated to occur in the general reproductive population in 10–15% of clinically recognized pregnancies (US Department of Health and Human Services, 1982). Approximately 50% of such miscarriages are associated with cytogenetic abnormalities, with trisomy being the most frequent, followed by polyploidy and monosomy X (Lauritsen, 1975; Hassold, 1986; Kalousek *et al*., 1993). Such miscarriages are thought to occur on a random basis, with an increasing frequency of trisomy with advancing maternal age (Hassold and Chiu, 1985). More than 99% of chromosomally abnormal pregnancies result in miscarriage, most of which occur prior to 10 weeks gestation (Jacobs and Hassold, 1987).

Recurrent miscarriage, defined as three or more consecutive miscarriages, affects up to 3% of couples trying to establish a family (US Department of Health and Human Services, 1982). Risk factors for recurrent miscarriage include: loss of a euploid pregnancy, loss after the first trimester, difficulty conceiving, and delivery of a very low birthweight baby (Strobino *et al*., 1986). Historically, structural genetic, endocrine, anatomic and autoimmune factors are associated with recurrent miscarriage in ~60% of cases (Hill *et al*., 1992; Clifford *et al*., 1994; Stephenson, 1996). In the other 40% of cases, no association with these factors could be found. Routine cytogenetic analysis of miscarriages has remained an uncommon practice to date. This unfortunate omission has impacted on the management of couples with repeated pregnancy wastage. Not only has cytogenetic analysis of miscarriages been shown to be cost-effective in the health care system (Wolf and Horger, 1995), but the results also help the physician to decide whether further investigations are warranted. In addition, the results are useful in the counselling of couples who are trying to understand why their pregnancy ended in miscarriage and to decide whether to try again.

The objectives of this study were to determine the frequency and distribution of cytogenetically abnormal miscarriages from couples with recurrent miscarriage and to compare the results with the general population using historical data. Our hypothesis was that euploid miscarriages should be more common in couples with recurrent miscarriage, a finding that would support an association between non-cytogenetic factors and recurrent miscarriage. In addition, the distribution of cytogen-

etic abnormalities in miscarriages from couples with recurrent miscarriage should be similar to the general reproductive population since these errors are thought to occur randomly, although frequency increases with advancing maternal age (Warburton *et al*., 1987).

Materials and methods

Populations

Patients with a history of recurrent miscarriage, with at least one miscarriage that had been sent for cytogenetic analysis, were identified using a Recurrent Pregnancy Loss (RPL) Database (Access '97) developed by the author (M.D.S.). University and institutional ethics approval had been obtained. The gestational age at time of pregnancy demise was estimated by reviewing ultrasound and/or embryo pathology reports. If neither was available, the gestational age was based on symptomatology. The miscarriages were grouped into pre-clinical loss (demise $<$6 weeks gestation), embryonic loss (demise at $\geqslant$6 weeks but $<$10 weeks gestation) and fetal loss (demise at $\geqslant$10 weeks but $<$20 weeks gestation). The maternal age at time of pregnancy demise was determined. If there was a twin gestation, both specimens were reported separately although the miscarriage was considered a single event.

The frequency and distribution of cytogenetically normal and abnormal miscarriages were compared with data (n = 7182) compiled from seven miscarriage surveys done in different geographic areas (Jacobs and Hassold, 1987) and selected specimens (n = 1228) from the same institution (Kalousek *et al*., 1993). The recurrent miscarriage data were stratified for maternal age and compared with stratified population-based data (n = 2201) (Hassold and Chiu, 1985).

Cytogenetic analysis

Culturing of amniotic membrane and/or chorionic sac followed by routine cytogenetic analysis using Giemsa banding was used exclusively until the year 2000. Subsequently, comparative genomic hybridization (Lomax *et al*., 2000) was requested if the tissue failed to grow or if maternal contamination was questioned, i.e. cultured chorion revealed 46,XX.

Statistical analysis

Distributions were compared using the χ^2-test. In Tables II and III, the last two cytogenetic categories (structural and other) were collapsed for statistical analysis. Yates' correction for continuity was applied for 2×2 comparisons. $P < 0.05$ was accepted as statistically significant.

Results

A total of 320 patients with recurrent miscarriage with at least one miscarriage sent for cytogenetic analysis was identified between 1992 and 2000. A total of 472 miscarriages was sent for cytogenetic analysis. Of these, 325 specimens were obtained by dilatation and curettage (D&C) and 89 specimens were collected by the patients following spontaneous passage of tissue per vagina (expectant management). Cytogenetic analysis was unsuccessful in 58 miscarriages from 35 patients, of which 28/325 (8.6%) specimens were obtained by D&C and 30/89 (33.7%) following expectant management. The cytogenetic failure rate was significantly higher if the specimen was collected with spontaneous passage of tissue (P = 0.000002). Excluding those with no cytogenetic results, the data set to be analysed consisted of 285 patients with recurrent miscarriage. There were six sets of diamniotic twins, therefore there were 420 specimens from 414 miscarriages that had successful cytogenetic analyses.

Table I. Frequency of cytogenetic diagnoses in 420 miscarriages from 285 couples with recurrent miscarriage

Diagnosis	No. of miscarriages	Frequency (%)
Euploid, female[a]	120	29
Euploid, male[b]	105	25
Trisomy 1	0	0
Trisomy 2	4	0.95
Trisomy 3	0	0
Trisomy 4	1	0.24
Trisomy 5	1	0.24
Trisomy 6	3	0.7
Trisomy 7	3	0.7
Trisomy 8	4	0.95
Trisomy 9	4	0.95
Trisomy 10	1	0.24
Trisomy 11	1	0.24
Trisomy 12	1	0.24
Trisomy 13	11	2.6
Trisomy 14	11	2.6
Trisomy 15	22	5.2
Trisomy 16	19	4.5
Trisomy 17	2	0.48
Trisomy 18	4	0.95
Trisomy 19	0	0
Trisomy 20	2	0.48
Trisomy 21	11	2.6
Trisomy 22	16	3.8
Double trisomy	9	2.1
Sex trisomy (47,XXY)	1	0.24
Monosomy X (45,X)	18	4.3
Monosomy X and trisomy 21	1	0.24
Triploidy	27	6.4
Tetraploidy	10	2.4
Unbalanced translocations	8	1.9
Total	420	100

[a]Consisting of 118 cases of 46,XX and two cases of balanced translocations.
[b]Consisting of 105 cases of 46,XY.

The 285 patients in the data set had a total of 1281 documented miscarriages. Therefore, the median number of miscarriages per patient was four (range 3–12). The mean maternal age at time of pregnancy loss was 34.3 years (range 19–46 years). The miscarriages were pre-clinical in 29/420 (7%), embryonic in 237/420 (56%) and fetal in 154/420 (37%).

The frequency of cytogenetic diagnoses in the recurrent miscarriage data set is summarized in Table I. In all, 225 (54%) of the specimens that had a cytogenetic diagnosis were euploid, of which 120 were female (including two balanced translocations) and 105 were male. A total of 195 (46%) of the specimens were abnormal, of which 131 (67%) were trisomic, 37 (19%) were polyploid, 18 (9%) were monosomic X and eight (4%) were unbalanced translocations, either reciprocal or Robertsonian. There was one case of combined trisomy 21 and monosomy X (46,X+21). The most frequent trisomic was 15 (22 cases), followed by trisomies 16 (19 cases), 22 (16 cases), 21 (12 cases), 14 (11 cases) and 13 (11 cases).

The distribution of cytogenetic categories, stratified for gestational age groups, is shown in Table II. Although there was an excess of females within the euploid category, the

Table II. Distribution of miscarriages according to cytogenetic categories, stratified by gestational age groups

	Total	Euploid	Trisomic[a]	Monosomy X	Polyploidy	Structural[b]	Others
Pre-clinical (<6 weeks)	29	17[c] (59)	8 (28)	0	2 (7)	2 (7)	0
Embryonic (6–10 weeks)	237	119[d] (50)	83 (35)	9 (4)	20 (8)	5 (2)	1 (0.4)
Fetal (⩾10–20 weeks)	154	89[e] (58)	40 (26)	9 (6)	15 (10)	1 (0.7)	0
Total	420	225 (54)	131 (31)	18 (4)	37 (9)	8 (2)	1 (0.2)

Values in parentheses are percentages.
[a]Including mosaic trisomies, sex trisomies and double trisomies.
[b]Unbalanced translocations.
[c]Consisting of nine cases of 46,XX, and eight cases of 46,XY.
[d]Consisting of 64 cases of 46,XX (including two cases of balanced translocations), and 55 cases of 46,XY.
[e]Consisting of 47 cases of 46,XX, and 42 cases of 46,XY.

Table III. Comparison of distribution of miscarriages according to cytogenetic categories

	Total	Euploid[a]	Trisomic[b]	Monosomy X	Polyploidy	Structural[c]	Others
Recurrent miscarriage	420	225 (54)	131 (31)	18 (4)	37 (9)	8 (2)	1 (0.2)
Unselected populations[d]	7182	3738 (52)	1923 (26.8)	615 (8.6)	709 (10)	147 (2)	50 (0.7)
Selected population from same institution[e]	1228	457 (37)	417 (34)	101 (8.2)	183 (15)	70 (6)	0

Values in parentheses are percentages.
[a]Consisting of 46, XX, 46, XY and balanced translocations.
[b]Including mosaic trisomies, sex trisomies and double trisomies.
[c]Unbalanced translocations.
[d]Seven surveys tabulated by Jacobs and Hassold (1987).
[e]Kalousek *et al*. (1993).

excess was not statistically significantly different from 50:50 within or between gestational age groups. There was no significant difference found in the distribution of cytogenetically normal and abnormal miscarriages in the three gestational age groups, using a 2×3 table. In addition, there was no significant difference found in the distribution of cytogenetically abnormal categories, using a 4×3 table.

In Table III, the distribution of cytogenetic categories in the recurrent miscarriage data set was first compared with the tabulated data set of seven miscarriage surveys (Jacobs and Hassold, 1987). There was a significant difference in the distribution of cytogenetic categories between the two data sets ($P = 0.01$), with a trend towards more trisomics (31 versus 26.8%) and less monosomy X (4 versus 8.6%) in the recurrent miscarriage group. Secondly, the recurrent miscarriage data set was compared with a selected cytogenetic data set from the same institution between 1978 and 1989, based on a reproductive history of recurrent pregnancy loss, advanced maternal age, or abnormal embryonic phenotype (Kalousek *et al*., 1993). A significant difference in the distribution of cytogenetic categories between the two data sets ($P = 0.0001$) was seen, with a trend towards more euploid specimens (54 versus 37%) and less monosomy X (4 versus 8.2%), polyploidies (9 versus 15%) and unbalanced translocations (2 versus 6%).

The frequency of euploid specimens in the recurrent miscarriage data set, stratified for maternal age at time of pregnancy loss, was compared with a population-based data set (Hassold and Chiu, 1985), as shown in Table IV. The Hassold and Chiu data were used for this comparison since the larger Jacobs and Hassold study did not stratify for maternal age. More euploid specimens were observed in women with recurrent miscarriage aged 18–29 years (65 versus 52%, $P = 0.03$) and aged 30–35 years (63 versus 48%, $P = 0.001$) than in the unselected population. There was no difference in the women aged ⩾36 years.

The distribution of cytogenetically abnormal specimens in the recurrent miscarriage data set, stratified for maternal age at time of pregnancy loss, was compared with the same population-based data set (Hassold and Chiu, 1985), as shown in Table V. No significant difference in such distribution was found in any of the age groups.

Discussion

Recurrent miscarriage continues to be a challenging reproductive problem for the patient and clinician. Identifying a cytogenetic cause for a miscarriage can be psychologically important to overcome grief and loss, as well as deciding on whether to try again. Cytogenetic analyses of previous miscarriages are an important component in the assessment of

Table IV. Comparison of euploid miscarriages stratified by maternal age at time of miscarriage

Age (years)	Comparative groups	No. of miscarriages	Euploid	*P*
18–29	Recurrent miscarriage	84	55 (65)	0.03
	Unselected population[a]	1304	684 (52)	
30–35	Recurrent miscarriage	150	95 (63)	0.001
	Unselected population[a]	637	306 (48)	
36–39	Recurrent miscarriage	113	49 (43)	NS
	Unselected population[a]	185	76 (41)	
⩾40	Recurrent miscarriage	73	26 (36)	NS
	Unselected population[a]	75	20 (27)	
Total	Recurrent miscarriage	420	225 (54)	NS
	Unselected population[a]	2201	1086 (49)	

Values in parentheses are percentages.
[a]Data from Hassold and Chiu (1985).
NS = not significant.

Table V. Comparison of cytogenetically abnormal miscarriages stratified by maternal age at time of miscarriage

Age (years)	Comparative groups	Cytogenetically abnormal miscarriage	Trisomic	45,X	Polyploid	Others[a]	*P*
18–29	Recurrent miscarriage	29	16 (55)	5 (17)	8 (28)	0 (0)	NS
	Unselected population[b]	620	284 (45)	147 (24)	135 (22)	54 (9)	
30–35	Recurrent miscarriage	55	27 (49)	7 (13)	18 (33)	3 (5)	NS
	Unselected population[b]	331	219 (66)	39 (12)	62 (19)	11 (3)	
36–39	Recurrent miscarriage	64	45 (70)	6 (9)	8 (13)	5 (8)	NS
	Unselected population[b]	109	81 (74)	16 (15)	6 (5.5)	6 (5.5)	
⩾40	Recurrent miscarriage	47	43 (91)	0 (6)	3 (2)	1	NS
	Unselected population[b]	55	50 (91)	1 (2)	3 (5)	1 (2)	
Total	Recurrent miscarriage	195	131 (67)	18 (9)	37 (19)	9 (5)	0.008
	Unselected population[b]	1115	634 (57)	203 (18)	206 (18)	72 (6)	

Values in parentheses are percentages.
[a]Including unbalanced translocations.
[b]Data from Hassold and Chiu (1985).
NS = not significant.

couples with a history of recurrent miscarriage. A recent manuscript described cytogenetic analyses of formalin-fixed, paraffin-embedded miscarriage specimens using comparative genomic hybridization (Bell *et al*., 2001). This new application of comparative genomic hybridization allows the clinician to request retrospectively cytogenetic information about prior pregnancy losses in couples with a history of recurrent miscarriage. If prior miscarriages are cytogenetically abnormal, further evaluation may not be warranted, resulting in time-savings to both the couple and the clinician, as well as cost-savings to the health care system.

Although this is a large study comparing the cytogenetic diagnoses of miscarriages in couples with recurrent miscarriage to an unselected reproductive population and a selected population from the same institution, further studies are warranted. Since comparative genomic hybridization was not introduced clinically until recently, some of the published control data may contain erroneous results due to culturing of tissue contaminated by maternal cells. In addition, this technique may also help to reduce bias caused by culture failure, which may be more common in chromosomally abnormal pregnancy tissue. With the advancement of early pregnancy monitoring, the historic distribution of cytogenetic results may differ significantly from recent results because of the inclusion, although limited, of pre-clinical loss. Collection of matched control data from the same institution will be required to confirm these observations.

The significant difference in the failure rate of conventional cytogenetic analysis, according to mode of ascertainment of specimens, is an important clinical finding which has not

previously been reported. Unless comparative genomic hybridization is readily available, a D&C is advantageous over collection of spontaneously expelled tissue, if cytogenetic analysis is important for subsequent patient management.

The recent manuscript of Carp *et al*. reports the cytogenetic results of 125 specimens from 167 patients with unexplained recurrent miscarriage collected at curettage using conventional G-banding techniques (Carp *et al*., 2001). Their failure rate of 25% was significantly higher than ours of 12% overall ($P = 0.0001$). Carp *et al*. reported that 89 (71%) of the specimens were euploid, although the ratio of 46,XX and 46,XY was not included in their results. Their higher frequency of euploid miscarriages (71 versus 53%, $P = 0.0005$) could have been due to the subgroup of recurrent miscarriage patients studied or maternal contamination, or, most likely, the preferential culture failure of chromosomally abnormal specimens. The mean maternal age in their study was 31 years, slightly younger than in the present study, and thus would be expected to show a higher rate of euploid miscarriages.

As noted, the distribution of cytogenetically abnormal categories, stratified by maternal age at time of miscarriage, was not significantly different for the recurrent miscarriage data set and the unselected reproductive population of Hassold and Chiu (1995). Overall, the most frequent trisomic in the recurrent miscarriage group was 15, followed by 16, 22, 21, 14 and 13. In the large unselected data set of Jacobs and Hassold, trisomy 16 was the most frequent trisomic, followed by 22, 21, 15 and 13 (Jacobs and Hassold, 1987). Since the frequency of trisomics involving acrocentric chromosomes (13–15, 21, 22) is known to increase more strongly with maternal age than other trisomics (Hassold and Jacobs, 1984), the difference in trisomic frequency may simply reflect a higher mean maternal age in our data set. Alternatively, there may be significant between-laboratory variation in the detection of individual trisomies (Hassold *et al*., 1984). Lastly, the inclusion of cytogenetically defined pre-clinical miscarriages (demise <6 weeks gestation) in our data set could also have been responsible for the difference in trisomic frequency between the cases and controls.

The higher frequency of euploid miscarriages in women with recurrent miscarriage <36 years of age compared with the unselected reproductive population confirms that there may be other factors associated with recurrent miscarriage. This is consistent with the findings of Warburton *et al*. showing an increased risk for having a chromosomally normal miscarriage after one chromosomally normal miscarriage, once age of the mother was corrected for (Warburton *et al*., 1987). Only by excluding miscarriages with cytogenetic abnormalities will we be able to truly identify couples who have non-random recurrent miscarriage. The effect of maternal age in couples with recurrent miscarriage is significant. It is therefore even more important to determine the karyotype of losses in women aged $\geqslant 36$ years to avoid unnecessary, costly and time-consuming investigations and treatments. In addition, the effectiveness of therapeutic interventions may be more conclusively determined if index miscarriages that are cytogenetically abnormal are excluded from analysis (Ogasawara *et al*., 2000).

This study illustrates the importance of cytogenetic analyses of miscarriages in couples with a history of recurrent miscarriage. The results provide further evidence that there are non-cytogenetic factors associated with recurrent miscarriage, particularly in women under the age of 36 years. With widespread use of cytogenetic analysis by conventional G-banding and comparative genomic hybridization, when maternal contamination is questioned or with culture failure, the criteria used for evaluation of reproductive loss may change in the near future. We propose that a history of at least two euploid miscarriages, rather than the present criteria of at least three miscarriages (usually of unknown cytogenetics), would identify couples who are more likely to have a non-cytogenetic factor associated with their history of recurrent pregnancy loss. Such stringent cytogenetic criteria may also improve our ability to identify causative factors and therapeutic interventions that could be of benefit. With the storage of formalin-fixed miscarriage tissue, the clinician could selectively request comparative genomic hybridization to determine whether the couple met such criteria for evaluation. Patients and clinicians would benefit psychologically and diagnostically, as well as the health care system, from knowing which pregnancies were lost due to random meiotic errors and which couples required evaluation for non-cytogenetic factors associated with recurrent pregnancy loss.

Acknowledgements

The authors thank V.Poposka, MD for assisting with the data collection and T.Pacheco, CCHRA(C) for data entry and processing. We appreciate the cooperation of D.Kalousek, MD and the Cytogenetics Lab at British Columbia's Children's Hospital. Funding was provided by the Medical Research Council of Canada.

References

Bell, K.A., Van Deerlin, P.G., Feinberg, R.F., du Manoir, S. and Haddad, B.R. (2001) Diagnosis of aneuploidy in archival, paraffin-embedded pregnancy-loss tissues by comparative genomic hybridization. *Fertil. Steril*., **75**, 374–379.

Carp, H., Toddler, V., Aviary, A., Danielle, M., Mashiach, S. and Barkai, G. (2001) Karyotype of the abortus in recurrent miscarriage. *Fertil. Steril*., **75**, 678–682.

Clifford, K., Flanagan, A.M. and Regan, L. (1994) An informative protocol for the investigation of recurrent miscarriage; preliminary experience of 500 consecutive cases. *Hum. Reprod*., **9**, 1328–1332.

Hassold, T. (1986) Chromosome abnormalities in human reproductive wastage. *Trends Genet*., **2**,105–110.

Hassold, T. and Jacobs, P. (1984) Trisomy in man. *Annu. Rev. Genet*., **18**, 69–97.

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

Hassold, T., Warburton, D., Kiln, J. and Stein, Z. (1984) The relationship of maternal age and trisomy among trisomic spontaneous abortions. *Am. J. Hum. Genet*., **36**, 1349–1356.

Hill, J.A., Polgar K., Harlow B.L. *et al*. (1992) Evidence of embryo and trophoblast toxic cellular immune response(s) in women with recurrent spontaneous abortion. *Am. J. Obstet. Gynecol*., **166**, 1044–1052.

Jacobs P.A. and Hassold T.J. (1987) Chromosome abnormalities: origin and etiology in abortions and live births. In Vogal, F. and Sperling, K. (eds), *Human Genetics*. Springer-Verlag, Berlin, pp. 233–244.

Kalousek, D.K., Pantzar, T., Tsai, M. *et al*. (1993) Early spontaneous abortion: morphologic and karyotypic findings in 3,912 cases. *Birth Defects*, **29**, 53–61.

Lauritsen, J.G. (1975) The significance of oral contraceptives in causing chromsome anomalies in spontaneous abortions. *Acta Obstet. Gynecol. Scand*., **54**, 261–264.

Lomax, B., Tang, S., Separovic, E. *et al*. (2000) Comparative genomic hybridization in combination with flow cytometry improves results of cytogenetic analysis of spontaneous abortions. *Am. J. Hum. Genet*., **66**, 1516–1521.

Ogasawara, M., Aoki, K., Okada, S. *et al*. (2000) Embryonic karyotype of abortuses in relation to the number of previous miscarriages. *Fertil. Steril*., **73**, 300–304.

Stephenson, M.D. (1996) Frequency of factors associated with habitual abortion in 197 couples. *Fertil. Steril*., **66**, 25–29.

Strobino, B., Fox, H.E., Kline, J. *et al*. (1986) Characteristics of women with recurrent spontaneous abortions and women with favorable reproductive histories. *Am. J. Public Health*, **76**, 986–991.

US Department of Health and Human Services (1982) Reproductive impairment among married couples. In *US Vital and Health Statistics Series 23*, **11**, National Center of Health Statistics, Hyattsville, 5 pp.

Warburton, D., Kline, J., Stein, Z., Hutzler, M., Chin, A. and Hassold, T. (1987) Does the karyotype of a spontaneous abortion predict the karyotype of a subsequent abortion? Evidence from 273 women with two karyotyped spontaneous abortions. *Am. J. Hum. Genet*., **41**, 463–483.

Wolf, G.C. and Horger, E.O. (1995) Indications for examination of spontaneous abortion specimens: a reassessment. *Am. J. Obstet. Gynecol*., **173**, 1364–1368.

Submitted on June 15, 2001; resubmitted on September 10, 2001; accepted on October 30, 2001