Intrauterine death: an approach to the analysis of genetic heterogeneity

E BOTTINI, L COROMALDI, E CARAPELLA, R PASCONE, M NICOTRA, I COGHI, N LUCARINI, AND F GLORIA-BOTTINI

From the Interdisciplinary Centre for Applied Mathematics, Lincei National Academy, Rome; the Department of Genetics, University of Camerino, Camerino; and the Departments of Child Health and Obstetrics and Gynaecology, University of Rome, Rome, Italy.

SUMMARY A study of survival time of zygotes in utero and the relationship with parental phenotype of a series of genetic polymorphisms was carried out in 41 couples with habitual abortion. Variability of intrauterine survival time was found to be much higher between families than within families suggesting that several genetic entities contribute to the condition clinically defined as habitual abortion. Significant differences of survival time were found in relation to the length of the paternal Y chromosome and to the maternal phenotypes of PGM₁ and Ss. These observations are in line with previous data suggesting intrauterine selection in these polymorphisms. Further studies of the timing of intrauterine death in relation to 'normal' genetic polymorphisms may help to clarify the aetiology of spontaneous fetal loss.

Recent studies indicate that the overall death rate of zygotes ranges between 25 and 75%.^{1,2} Besides environmental agents, genetic factors are considered to be of paramount importance in this phenomenon.²⁻⁵ As in other phases of life, it is likely that both monogenic and polygenic heredity play an important part in intrauterine survival. Genetic factors may also play a role in predisposing to chromosomal aberration frequently observed in clinically recognisable spontaneous abortion.³

It is well known that genotypes expressing their deleterious effects after birth show a characteristic chronological pattern of manifestations. The timing of clinical manifestations and death is usually different between genotypes and very similar within families. By analogy, the study of survival time of zygotes in women with repeated abortion not resulting from anatomical or other known environmental causes, and the analysis of relationships with genetic factors statistically associated with fetal death, may improve the understanding of the aetiology of intrauterine death.

Material and methods

Forty-one couples who had had at least two consecutive abortions were examined. A total of 140 abortions was recorded.

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In general, after two consecutive abortions, the following examinations are carried out: hysterosalpingography, study of the cycle with the registration of the basal temperature and the serial determination of serum progesterone levels during the luteinic phase, glucose tolerance test, serological tests for lues, and toxoplasma tests. At least three examinations of the seminal fluid are carried out as well.

Tests for thyroid function, endometrium biopsies, and tests for cytomegalovirus, mycoplasmas, and listeria are carried out only in selected cases. Psychological investigations and help (frequent visits and availability at all times) are offered to all couples with habitual abortions. In the couples included in the present analysis all these clinical examinations were consistently negative.

Gestational duration of the first aborted fetus was always estimated from the date of the last menstrual period as recorded from the patient's memory. For subsequent miscarriages, ovulation date was generally determined by basal temperature charts.

In all couples a karyotype analysis was carried out with C or G banding⁶ or both and the phenotype of the following systems was determined by standard techniques: ABO, Rh, MNSs, Secretor (Se), haptoglobins (Hp), phosphoglucomutase (PGM₁), adenosine deaminase (ADA), and adenylate kinase (AK₁).⁷ The HLA phenotype (nine antigens for the A locus and 15 antigens for the B locus) was

TABLE Gestational duration in habitual abortion. Relation with parental phenotype of some genetic polymorphisms.

Variable	Category	All aborted fetuses				First aborted fetus			
		No	Mean gestation (weeks)	SD	Significance of difference	No	Mean gestation (weeks)	SD	Significance of difference
Total population		140	11.42	4.26	—	—	—	—	—
Long Y chromosome in the father*	Present	23	14.1	4.8	<0.001	7	16.3	6.6	<0.001
	Absent	117	10.9	4.0		34	9.5	3.0	
Maternal PGM genotype	Homozygote	99	10.8	4.2	<0.01	29	9.8	4.0	<0.05
	Heterozygote	41	12.9	4.2		12	12.9	5.3	
Maternal ADA genotype	Homozygote	133	11.4	4.3	NS	—	—	—	—
	Heterozygote	7	11.0	2.6		—	—	—	
HLA-Bw35 antigen in the mother	Present	52	10.9	3.5	NS	—	—	—	—
	Absent	66	11.9	4.6	(<0.20)	—	—	—	
HLA-A antigens of the couple	Sharing the same antigen	34	12.3	3.1	NS	—	—	—	—
	Not sharing the same antigen	75	10.8	4.4	(<0.10)	—	—	—	
HLA-B antigens of the couple	Sharing the same antigen	40	10.5	3.8	NS	—	—	—	—
	Not sharing the same antigen	64	11.7	4.3	(<0.20)	—	—	—	
Maternal Ss genotype	SS	25	13.4	5.3	<0.01	9	11.7	4.9	NS
	Others	115	11.0	3.9		32	10.4	4.5	
Paternal Ss genotype	SS	25	10.0	2.9	NS	—	—	—	—
	Others	115	11.7	4.4	(<0.10)	—	—	—	
Maternal Secretor phenotype	Not Secretor	70	11.3	4.1	NS	—	—	—	—
	Secretor	70	11.6	4.4		—	—	—	
Paternal Secretor phenotype	Not Secretor	77	11.0	4.6	NS	—	—	—	—
	Secretor	63	12.0	3.8	(<0.20)	—	—	—	
Wife-husband ABO incompatibility	Present	57	11.1	3.8	NS	—	—	—	—
	Absent	83	11.7	4.6		—	—	—	

*Criterion adopted for defining a Y chromosome as long was a ratio Y/F ≥ 1.0 .

determined in 35 couples. Preliminary data concerning the phenotype distribution of some of these systems have been published elsewhere.⁸⁻¹¹

Variables included in the present analysis are reported in the table. Selection of variables and their subdivision into categories was based on statistical association with spontaneous abortion observed in our sample or reported by other investigators.⁸⁻¹⁴ Statistical analyses were carried out using the SPSS programme with a 370/158 IBM computer.¹⁵

Results

The distribution of the mean gestational duration calculated for each couple with habitual abortion is depicted in the figure. Couples were quite uniformly distributed over the range observed in our sample. Variability of intrauterine survival time was much higher between families than within families. Variance analysis showed significant heterogeneity among couples.

The relation between gestational duration and some parental genetic variables is analysed in the table. Significant differences in intrauterine survival time were observed in relation to the length of the paternal Y chromosome and maternal PGM₁ and Ss phenotypes. The same pattern of associations was observed after examination of only the first aborted fetus of the couples.

Variance analysis showed no interaction between maternal PGM₁ phenotype, maternal Ss phenotype,

FIGURE Distribution of mean duration of gestation (weeks) in couples with habitual abortion.

Variance analysis. Total number of abortions = 140

	Mean square	df
Between families	31.3363	40
Within families	12.8355	99
F = 2.4414, p < 0.01.		

and length of Y chromosome in the father in their effects on gestational duration of aborted fetuses.

Discussion

The distribution of the mean gestational durations and the heterogeneity between families are compatible with the conjecture that several genetic entities contribute to the condition clinically defined

as habitual abortion. 'Normal' genetic polymorphisms seem at present to be the most interesting candidates for the analysis of multifactorial variables influencing intrauterine development and survival.¹⁶

Association with spontaneous abortion has been observed for ABO,¹² HLA,^{8, 13} PI,¹⁷ MNSs,⁹ PGM₁,¹⁰ Secretor (Bottini *et al*, unpublished observations), ADA,¹¹ and chromosomal variants.¹⁴ The observation of a relationship between gestational duration and a given polymorphism reinforces the statistical inference based on the increased proportion of a particular phenotype in spontaneous abortion. If a given genotype predisposes to fetal loss, a temporal pattern of recurrence could be associated with that genotype. The relevant phenomenon is the existence of a temporal distribution of fetal losses different from that observed among other genotypes. A decrease of intrauterine survival in comparison to other lethal conditions would not necessarily be expected.

The present analysis supports the view that PGM₁, Ss, and structural variation of the Y chromosome influence intrauterine selection and survival.

Our approach has not shown significant results for ADA, HLA, ABO, and Secretor. Several hypotheses for this can be put forward. (1) Statistical association previously reported was fortuitous, (2) the size of the present sample is not sufficient to reveal slight differences of gestational duration, and (3) these factors exert a protective action during the whole span of gestation and therefore lack a typical temporal pattern of manifestations.

The selection of couples with repeated abortions may have definite advantages for our study. While the correct assignment of a single episode of abortion as spontaneous or induced is generally difficult, our cases are certainly all spontaneous and it is likely that our sampling method selects families genetically predisposed.

The exact ascertainment of gestational duration is difficult; this, however, should not affect the results of our analysis since the error is likely to be independent of the genetic marker investigated. The fact that the pattern of relationships between the gestational durations and the polymorphisms was the same when considering only the first aborted fetuses (for which the exact ascertainment of gestational duration was more difficult) supports this view.

Although many more families should be studied in order to draw definite conclusions, our observations suggest that the analysis of genetic polymorphisms

in relation to the timing of fetal death may give insight into the aetiology of habitual abortion and into the identification of the forces which maintain genetic variability.

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Correspondence and requests for reprints to Dr E Bottini, Interdisciplinary Centre for Applied Mathematics, Accademia Nazionale dei Lincei, Rome, Italy.