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Reproductive Histories in a Norwegian Twin Population: Evaluation of the Maternal Effect in Early Spontaneous Abortion

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CONTENTS

Thesis submitted in partial fulfillment of the requirements for the PhD degree in the Virginia Commonwealth University, Richmond, 1980. Major Professor: Walter E. Nance.

An evaluation of the maternal effect on spontaneous abortion and the genetic contribution to various reproductive variables was made using like-sexed twins born between 1915–1946. Health and reproductive questionnaires were sent to 2,365 twin pairs listed in the Norwegian Twin Registry. Zygosity was determined by a questionnaire which proved to be 98% accurate in discriminating between monozygotic (MZ) and dizygotic (DZ) twins. Verification of the zygosity questionnaire was accomplished by blood cell marker zygosity determination in 200 twin pairs. Data from 428 monozygotic twin pairs (174 male pairs and 254 female pairs) were analyzed by a new model for qualitative traits and a striking maternal effect was found. The results suggested that over 54% of all women may belong to a high risk group who have approximately a 13% risk of early fetal loss. The incidence of first trimester abortions and spontaneous abortion reported by a sample of 915 like-sexed twin pairs and spouses was .079 and .089, respectively; there were no significant differences between male and female pairs.

Other reproductive variables were studied in both MZ and DZ twins including the age of menarche and menopause, the age at marriage and birth of the first child, and the interval between marriage and the first birth. These variables were examined for evidence of genetic effects and secular trends. The mean age of menarche had decreased significantly between the years 1915–1935 and 1936–1946. The secular trend appeared to be environmental in nature and accounted for more than 7% of the overall variation in age of menarche. Sixty-five percent of the variation in the age of menarche was attributed to genetic factors. The mean age of marriage and the mean age at the first birth decreased significantly between 1915–1935 and 1936–1946. Twins born between 1936–1946 also reported fewer pregnancies than did twins born between 1915–1946, but this was statistically significant among MZ twins only; no significant difference in the frequency of spontaneous abortions was found between the 1915–1935 and the 1936–1946 groups.

Although there were occasional groups in which differences were statistically significant, no general trends were identified in comparing couples who reported a spontaneous abortion with those who did not. Traits compared were: the age of menarche, age of marriage, age at first birth, the interval between marriage and first birth and the mean present age of the twins and spouses. Couples reporting a first trimester spontaneous abortion had significantly more total pregnancies than those not reporting a spontaneous abortion. Unaffected pregnancies were also significantly more numerous in couples with a spontaneous abortion compared to those without, suggesting over-compensation for fetal loss.

A maternal effect in spontaneous abortion was indicated by the correlation coefficients found; significant positive correlations were found in both MZ and DZ females but not in males. For total pregnancies and unaffected pregnancies, MZ twins, both male and female, had significant correlation coefficients while DZ twins did not.

Key words: Abortion, Fetal Death, Twins, Reproduction, Questionnaires, Medical Genetics, Population Genetics, Menarche, Menopause, Marriage

1. INTRODUCTION

Maternal effects are operative when the female parent makes a contribution to her offspring that is over and above her nuclear genic contribution [101]. Such effects can arise by several routes including cytoplasmic inheritance, maternal nutrition (both pre- and postnatally), transmission of pathogens or antibodies (both pre- and postnatally), and by imitative behavior [39]. Whatever the route, in traits expressed phenotypically by both parent and offspring, the net effect is that the phenotype of the offspring is more similar to that of the female parent than the male parent. A classical method for detecting maternal effects in quantitative traits is to compare the resemblance between parents and offspring [39]. Another powerful way to detect maternal effects in quantitative traits focuses upon the offspring of monozygotic (MZ) twins [115]. The MZ kinship model takes advantage of the fact that within a MZ half-sib family there are individuals who share all, one-half, one-quarter, or none of their genes (Fig. 1). The MZ kinship model is generally used to estimate the genetic and environmental components of variation in quantitative traits; it is particularly effective in detecting maternal effects by contrasting the results of analyses of offspring contained in male and female twin kinships. If a trait has a significant maternal effect, the half-sib offspring of MZ female twins will be more similar than the half-sib offspring of MZ male twins. Maternal effects have been shown to be important in traits such as birthweight [31] and verbal IQ score [133].

A special problem exists when one studies certain discrete traits. Some discrete or qualitative traits cannot be expressed by the parents—for example, lethal congenital malformation or spontaneous abortion. The MZ kinship model, which makes use of offspring comparisons rather than parent–offspring comparisons, is well suited for analysis of these traits. The extension of the quantitative model to analyze qualitative traits was first suggested by Nance et al [115], and simulation studies were performed that indicated the model's accuracy [32]. For a discrete trait such as spontaneous abortion, it is expected that, if a maternal effect is a significant factor, then the reproductive histories of female MZ twins will be more similar than the reproductive histories of male MZ twins. The qualitative half-sib model was felt to be applicable to the analysis of maternal effects in early spontaneous abortion, and the frequency of occurrence is high enough to permit the detection of maternal effects in a sample of families that is not prohibitively large in size. Although, based on a rudimentary knowledge of biology, one might predict a strong maternal effect in spontaneous abortions, no systematic statistical study has been undertaken.

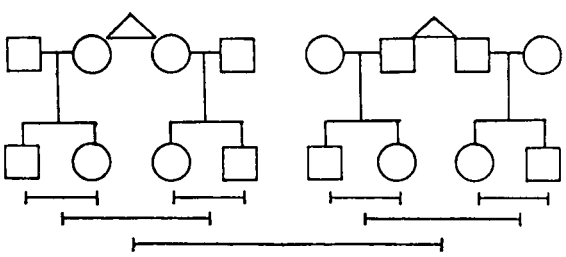

Fig. 1. Diagram showing structure of MZ kinship data. Lines below pedigree suggest how observed variation in offspring may be partitioned by a nested analysis of variance into among half-sibships, between sibships, and within sibship mean squares (after Nance et al, [115]).

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Data were collected by questionnaire from a population-based sample of twins contained within the Norwegian Twin Registry. In addition to the examination of maternal effects in early fetal loss, other reproductive variables such as age of menarche and menopause, age at marriage, and age at the birth of first child were studied for possible genetic influences and secular trends. Families were analyzed with respect to the absence or occurrence of spontaneous abortion; differences in the above reproductive variables as well as differences in the number of pregnancies were studied.

2. LITERATURE REVIEW

2.1. Definition and Incidence of Spontaneous Abortion

Spontaneous abortion, as defined by the World Health Organization (WHO), is the "non-induced separation from the mother of the product of conception before it is sufficiently mature to lead an independent life." Since estimates of the incidence of spontaneous abortions must be based upon recognizable cases, the true incidence of this event is difficult to assess. In general, records of spontaneous abortions are obtained from several sources including hospitals, genetic or fertility clinics, and private medical practices. Each source may have a particular bias. For example, fertility clinics would tend to report cases of individuals with repeated abortions more often than such cases are seen in general hospital records. On the other hand, an induced abortion may not be reported as such in the hospital emergency room and thus appear on the hospital record as a spontaneous abortion. Hospital records may also be deficient in cases of early spontaneous abortion. Records of spontaneous abortion ascertained through private obstetrical practices may contain the least selection bias: Private physicians generally follow their patients from early in the pregnancy until termination. The private physician's records include abortions occurring inside or outside the hospital, and these abortions may be first-time or repeat abortions. Roth [134] found an incidence of spontaneous abortion of 15.6% in a follow-up study of 3,549 pregnant women in his obstetrical practice. Most investigators use an incidence of 10–15% as a working estimate of the frequency of spontaneous abortion. The actual rate of the termination of conception is thought to be much higher [129]. In a recent study on postimplantation loss, human chorionic gonadotropin (hCG) levels in urine were used to determine pregnancy in 197 normal females [109]. Out of 623 cycles 152 conceptions were detected by hCG levels. A 43% pregnancy loss was reported; 9.2% of the losses were clinically recognizable spontaneous abortions, and 33% were diagnosed only by hCG levels. These data illustrate the magnitude of the possible error in estimates of the occurrence rate of spontaneous abortion. Additionally, inferences about primary mutation rates, disease frequencies, and teratogenic effects may be influenced by imprecise estimates of fetal loss.

Spontaneous abortions have been classified generally as either early (up to 16 weeks' gestation) or late (17–28 weeks' gestation), or have been described as first-, second-, or third-trimester abortions. The age of the fetus is approximated in several ways. The most common estimate, referred to as menstruational age, uses the first day of the last menstrual period as day 1 of gestation. Day 1 of ovulatory age is 14 days

after commencement of the last menstrual period. The crown–rump length of the fetus is also used to estimate the age, but in abortuses this is frequently impossible to measure.

2.2. Causes of Spontaneous Abortion

2.2.1. Chromosome abnormalities and factors that increase the risk of chromosome abnormalities. It is estimated that up to 60% of first-trimester spontaneous abortions result from recognizable chromosome abnormalities [81]. A wide variety of chromosomal abnormalities, representing nondisjunctional events at the time of gametogenesis causing trisomy or monosomy, errors at fertilization causing triploidy, and first-division errors of the fertilized egg leading to tetraploidy or mosaicism, are seen commonly in early abortions. The frequency with which these errors occur is unknown, but there is some information on the relative frequency of gametogenic errors in males and females, which will be discussed later. Structural chromosomal aberrations such as deletions, pericentric inversion, and translocations are seen less frequently in abortus material, either because the events leading to them are indeed less frequent or because of the technical difficulties involved in identifying small changes. Structural abnormalities may involve critical areas of the genome resulting in very early fetal loss, and consequently may not be found in abortion studies.

There are significant problems involved in collecting data on spontaneous abortions. Since abortion material usually is obtained from the same sources that provide data on incidence, the possible ascertainment biases discussed previously may be important with respect to estimates of the frequency of various chromosomal abnormalities among spontaneous abortion. Furthermore, technical problems such as the culturing of material that is frequently in poor condition and in vitro cell selection may influence the proportion of successfully cultured and karyotyped abortuses and therefore have an effect on the frequency at which specific chromosome aberrations are detected [15]. In spite of their limitations and difficulties, chromosomal studies on spontaneous abortions have added considerably to our knowledge concerning the etiology of early fetal loss. The use of chromosome banding methodology has further increased the detection of structural abnormalities and has more definitively identified the chromosomes involved in numerical abnormalities.

One of the first reports on the use of chromosome banding techniques in the study of aborted material was given by Kajii et al [69]. These investigators found chromosome abnormalities in 82 out of 152 (54%) abortuses ascertained through a maternity clinic. A variety of abnormalities were found, with trisomy 16 being the most frequently observed abnormality (Table 1). First- and second-trimester abortions differed little in the percentage of abnormal karyotypes detected (59% and 51%, respectively). One explanation of this minimal difference between first- and second-trimester abortuses could be the exclusion of abortuses with a crown–rump length of over 100 mm. Since the average fetus attains a crown–rump length of 100 mm by the first part of the second trimester [151], abortions occurring later in second trimester were excluded. As Creasy et al [34] have shown, the percentage of abortuses with abnormal karyotypes decreases as the second trimester progresses. It is reasonable to conclude that a truer estimate of the portion of the second-trimester abortuses having chromosomal abnor-

TABLE 1. Summary of Studies of Spontaneous Abortion Using Cytogenetic Techniques

Karyotype	Kajii et al [69]	Boué et al [15]	Creasy et al [34]	Lauritsen [80]
Total N	152	1498	941	255
Total abnormal	82 (53.9%)	921 (61.48%)	287 (30.5%)	140 (54.9%)
Total normal	70 (46.1%)	577 (32.52%)	654 (69.5%)	115 (45.1%)
Details of abnormals				
Monosomy total	13	141	69	40
X	12	140	69	40
21	1	1		
Specific trisomies				
Total* group A	2	12	11	1
1				
2	1		6	1
3	1		1	
Total group B	1	6	2	1
4	1		1	1
5				
Total group C	6	86	17	10
6	1			
7	1			2
8	1		3	3
9	1		3	3
10	1		1	2
Total group D	11	109	21	16
13			3	1
14	4		3	4
15	3		4	11
Total group E	20	172	60	27
16	18		35	21
17				
18			6	6
Total group F		7	3	
19				
20			2	
Total group G	9	87	29	6
21	5		10	
22	2		11	6
XXY	1			
XXX				2
Trisomy total	50	479	143	64
Double trisomy	1	16		1
Mosaic trisomy				1
Polyploidy	17	240	51	26
Triploidy		183	37	14
Triploidy +	2		2	
Tetraploidy	5	57	12	12
Structural abnormalities	3	35	10	
Miscellaneous			16	3

*Group totals include unspecified trisomies.

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malities would be lower than 51%. Thus the difference in occurrence of chromosome abnormalities between first- and second-trimester abortuses may be greater than Kajii et al estimated.

Boué and co-workers [15] karyotyped approximately 1,500 spontaneous abortions that occurred before the 13th week of gestation. Specimens were collected from hospitals or from the women themselves if the abortion occurred at home. Of 1,498 cases studied, 61% had an abnormal karyotype. The types of chromosome abnormalities and the frequency of their occurrence are shown in Table 1. The trisomies were the most frequently seen abnormality; the E-group chromosomes represented about 36% of the autosomal trisomies. The specific chromosomes involved in the trisomies were not identified other than by chromosome group. Only 3.8% of all abnormal cases involved structural rather than numerical abnormalities; however, Boué and co-workers felt that the structural abnormalities might be underrepresented. Fifty percent of the karyotypically normal abortuses had physical abnormalities that were similar to those found in the karyotypically abnormal group. Boué et al suggested that some of the malformed abortuses in the karyotypically normal group may have structural chromosomal rearrangements that could not be detected with their cytogenetic techniques. Advanced maternal age was shown to be a significant factor only in the cases of autosomal trisomy, where the mean age of the mother was 31.3 years as compared with 27.5 years in all other categories.

In 1976, Creasy and co-workers [34] reported on 941 abortuses ascertained from hospitalized cases. The frequency of abortuses with chromosomal abnormalities was 30.5%. The distribution of chromosome abnormalities was compatible with the other studies (Table 1). The lower overall frequency of chromosome abnormalities reported by Creasy and co-workers probably resulted from the inclusion of all spontaneous abortions regardless of gestational age. The proportion of chromosomally abnormal abortuses was found to decrease with increasing gestational age. The highest proportion (60%) was recorded at 11 weeks' gestation. More than 33% of the trisomies involved an extra chromosome 16. Monosomy for the X chromosome was the most frequently observed abnormality. An increased mean maternal age was found to be significant in the mothers of trisomic abortuses (30 years) as compared to chromosomally normal abortuses (26.4 years).

Lauritsen [80] reported on a comprehensive study of spontaneous abortion that included karyotyping of the abortus and parents, placenta pathology, and a follow-up study of subsequent pregnancies. All cases had been admitted to a hospital and were of 16 weeks' gestation or less. Chromosome abnormalities were found in 55% of the 255 abortus specimens. The distribution of abnormalities in this study group is given in Table 1. Trisomy 16 was again the most frequent of the trisomies reported; for the total sample, monosomy X was the most frequent abnormality observed. Four of the 525 parents karyotyped had a major chromosome abnormality such as a balanced translocation or trisomy X. Maternal age was found to be a significant factor in trisomy 15, whereas paternal age was a factor in trisomies 16 and 18. Chromosome aberrations were found in 61% of first-trimester abortuses compared to 44% of second-trimester abortuses. At least half of the 35 women who reported having had a viral infection during pregnancy had chromosomally abnormal abortuses. Other causes of spontaneous abortion found included direct trauma (8), cervical incompetence (4), atresia uteri (1), gonorrhea (1), and IUD complications (3), all of which had normal karyotypes. All but one of 81 consecutive cases studied had a specific pathologic le-

sion of the placenta; hydatidiform degeneration of chorionic villi was the most frequent lesion. No difference was found in the frequency of placental lesions between karyotypically normal and abnormal abortuses.

In a 1½–3½ year follow-up study, Lauritsen found that the prognosis for the subsequent pregnancies was correlated closely with previous reproductive history. In eight women two consecutive abortions were studied, and in each case the abortuses were either both chromosomally abnormal or both chromosomally normal. These findings were similar to the results of a study by Alberman et al [3] in which chromosome analysis was performed on two consecutive abortuses in 23 mothers. In 17 of these women, both abortuses were chromosomally normal; in two women, both abortuses were abnormal; and four abortuses were found to be discordant for chromosomal abnormalities. Although there was a tendency for recurrence of spontaneous abortion with the same etiology, this trend was not significant. In a larger series of cases, Boué and Boué [14] found a highly significant correlation in the etiology of abortions among 43 women with two consecutive abortions; however, the chromosomal abnormality was not invariably the same in pairs concordant for the existence of any chromosomal abnormality. Results of the studies by Alberman et al [3] and Boué and Boué [14], along with the results of Lauritsen [80] and Kajii and Ferrier [70], are pooled in Table 2. While there is inconsistency in specific chromosomal abnormalities in these studies as seen in abortuses, there is a significant concordance for the presence of a chromosomal abnormality.

Meiotic or mitotic nondisjunction is of major interest as a cause of spontaneous abortion, since the majority of chromosomally abnormal abortuses are either trisomic or monosomic. The mechanism of nondisjunction, its frequency, and its outcome have been studied in a variety of organisms including Drosophila, rodents, and plants [51]. Evidence from animal studies suggests that the relative frequency of nondisjunction is higher in oocytes than in spermatocytes [51]. Gropp et al [51], in a study of the gametogenic products of Robertsonian-like translocation heterozygotes in tobacco mice, found that the percentage of hypermodal and hypomodal chromosome constitutions at meiotic metaphases II was significantly higher in the females than in the males. Oshimura and Takagi [121], working with a similar system, examined the chromosome constitution in the progeny of a backcross. They found that, although the reduction in fertility was similar in male and female translocation heterozygotes, nondisjunctional errors accounted for a higher proportion of errors in females.

TABLE 2. Summary of Studies of Consecutive Abortions; Number of Pairs Indicated

	Second abortion					
First abortion	Normal	Monosomy	Trisomy	Polyploidy	Translocation	Total
Normal	34	2	6	5	0	47
Monosomy	3	1	2	1	0	8
Trisomy	4	1	20	1	0	26
Polyploidy	1	1	3	1	0	6
Translocation	0	0	0	0	4	4
Total	43	5	31	8	4	91

Source: Boué and Boué [14]: 43 pairs; Alberman et al [3]: 23 pairs; Lauritsen [80]: 7 pairs; Kajii and Ferrier [70]: 18 pairs.

Human studies have taken advantage of Q and C banding heteromorphisms to identify the parental origin of either the extra chromosome in trisomies or extra set of chromosomes in triploidy. In 22 cases of trisomy-16 abortuses, 11 had a C or Q banding heteromorphism in which parental origin could be determined. In 7 cases, the trisomy originated from nondisjunction during the first meiotic division in the mother. Two cases resulted from nondisjunction in the first meiotic division in the father. Finally, two cases arose from nondisjunction in the second meiotic division; one case occurred in the mother and the other in the father. These data, along with the results of studies of Down syndrome children in whom the extra chromosome 21 more frequently appears to be of maternal origin, indicate that nondisjunctional events are not randomly distributed but occur preferentially in the female parent [108]. Furthermore, it appears that, while first or second meiotic nondisjunction occurs with similar frequencies in males, an error in the first meiotic division is much more common in females. A summary of seven studies dealing with the origin of the extra chromosome 21 is shown in Table 3.

Triploidy can arise through several mechanisms including dispermy, fertilization of two fused ova, diploid sperm, and diploid ovum. In a study of 21 cases of triploidy, Jacobs and co-workers found that dispermy accounted for over 66% of the cases. Other smaller series have shown both maternal [66, 80] and paternal origin of the extra haploid set of chromosomes [102, 163].

There are many factors that might increase the risk of chromosome abnormalities given that any substance, infection, or condition that affects the chromosomes could be considered a potential cause of early fetal loss. Of the many theoretical genetic factors that may increase the risk of spontaneous abortion, significant correlations have been made between some classes of translocation heterozygotes and the occurrence of spontaneous abortion [22, 70, 149]. When couples are selected on the basis of "habitual abortion" (two, three, or more spontaneous abortions), the incidence of translocation heterozygotes was 31.2% [149]. Other studies have reported a statistically significant increase in the frequency of translocation heterozygotes [116, 169]. Generally, it is agreed that for couples with a history of recurrent abortion and/or a previous fetus with malformations, chromosome analysis on the parents is highly warranted [116].

Jacobs et al [61] measured reproductive fitness in 192 pedigrees with various structural abnormalities or variants. Reproductive fitness was estimated using four parameters, comparing carriers to controls: the numbers of live births, fetal deaths, offspring surviving to 18 years of age, and relative generation times. D/D Robertsonian translocation showed a significant decrease in relative reproductive fitness, male and female heterozygotes showing approximately equal decreases. D/G Robertsonian translocation

TABLE 3. Summary of Studies of the Origin of the Extra Number 21 Chromosome in Down Syndrome Children

Origin	First meiotic division	Second meiotic division	Unknown	Total
Maternal	17	7	3	27
Paternal	6	7	2	15
Total	23	14	5	42

Source: Robinson [131], Lindsten et al [92], Mutton [111], Sasaki and Hara [138], Uchida [162], Wagenbichler et al [165], and Mikkelsen et al [108].

heterozygotes differed from D/D Robertsonian translocation heterozygotes in that no significant decrease in reproductive fitness was shown, even though there was a significant decrease in the number of live-born offspring born to female translocation heterozygotes surviving to 18 years of age. Previous studies by Hamerton [53] have shown an increased incidence of aneuploid offspring born to D/G female translocation heterozygotes. Since aneuploidy is associated with an increased infant mortality, the decrease in survival among offspring of D/G Robertsonian translocation heterozygotes is not surprising. Reciprocal translocations showed a significant reduction in reproductive fitness, and, again, there was no difference between male and female heterozygotes. A marked difference between male and female carriers was seen in a group described as "ring, marker, and supernumerary chromosomes," in which males but not females had significantly reduced reproductive fitness. Jacobs and co-workers claimed that the effect was due primarily to the kinships containing individuals with supernumerary chromosomes, and suggested that this supported the previous findings of an excess of males with a supernumerary chromosome among individuals attending subfertility clinics [23]. Chandley's study [23] also noted that in 31 pedigrees the proband had a noticeable chromosomal variant involving either the heterochromatic region of chromosome 1, 9, or 16, or the short arm of the acrocentric chromosomes. Both male and female carriers of these variants had a significantly lower reproductive fitness. This is of interest since this is the only group that did not have a structural abnormality per se, but rather was characterized by a variant that is considered normal but which may influence reproductive ability [54].

Marker chromosomes such as enlarged satellites, enlarged or missing short arms in the acrocentrics, and enlarged secondary constrictions have often been implicated as a source of increased risk of nondisjunction, thus increasing the risk of spontaneous abortion [94]. Holbek et al [59] found a total frequency of 26.9% of maker chromosomes among 80 sets of parents with spontaneous abortion. Thirty-six couples had had a karyotypically abnormal abortus. No significant difference in the frequency of marker chromosomes was detected between the two groups (27.8% and 26.2%, respectively). Significant differences were found only when the type of marker was examined; secondary constrictions and large Y chromosomes were more frequent in the karyotypically abnormal group, whereas short arm and satellite variants were more frequent in the normal group. The association between the large Y chromosome and spontaneous abortion has been noted in several other studies [45, 117, 122]. Hamerton et al [55] found an association between an enlarged short arm in one of the acrocentric chromosomes and the occurrence of trisomy 21 in the offspring. One possible mechanism for the increased risk of spontaneous abortion associated with enlarged short arms or satellites of the acrocentric chromosomes is the increased degree of satellite association, which increases the risk of nondisjunction [54].

One hypothesis to explain the increased risk for chromosomally abnormal fetuses in some individuals postulates the existence of a gene that causes an increase in nondisjunction or other meiotic disturbances. Plant studies have provided some indirect evidence in support of this hypothesis. For example, Lesley and Frost [87] discovered a gene that causes a decrease in coiling of chromosomes and thus results in longer than normal chromosomes. In Matthiola incanca and barley, this phenomenon was associated with an increase in the production of trisomic progeny [87]. Clayberg [25] has described a gene in tomatoes that causes precocious centromere division during

meiosis, and results in the production of aneuploid progeny. Several genes have been identified that affect meiosis in Drosophila melanogaster. "Gowen's gene" is a third chromosome recessive that prevents crossing over and leads to a greatly increased frequency of nondisjunction [48]. In D. simulans, the mutant *claret* disturbs meiosis by distorting spindles at the first meiotic division, causing failure of the second division and cleavage irregularities [35]. A similar mutation has been observed in D. melanogaster: the ca^{nd} (*claret*-nondisjunction) gene, which is known to cause nondisjunction of the X and fourth chromosomes [35]. All of these Drosophila mutants are sex-limited, occurring exclusively during oogenesis. In humans, evidence supporting the existence of genic control of nondisjunctional disturbances is provided by the finding that some women repeatedly produce trisomic conceptuses. Alberman et al [3] noted that the occurrence of Down syndrome was ten times higher than expected in siblings with a mother presenting with a chromosomally abnormal abortus. A recent study of an inbred population in Kuwait suggested that consanguinity and maternal age, independently, were significant factors in the occurrence of Down syndrome [4]. These investigators reported 20 cases of trisomy 21 out of 11,614 singleton births in 1 year. The relative risk was estimated to be four times higher in closely related parents than in unrelated parents. Two explanations of the results were suggested: 1) that a gene exists inducing mitotic nondisjunction in the homozygous fertilized ovum, or 2) that an autosomal-recessive gene exists that results in meiotic nondisjunction in the homozygous parent. The authors justified their second explanation by stating that parents who are consanguineous tend likewise to be products of consanguineous matings, and therefore the probability of an autosomal-recessive homozygote would be greater than in unrelated parents.

Prior to the application of chromosome banding methodology, an association was noted between increasing maternal age and increasing birth order with an increased risk of spontaneous abortion. The reasons proposed for an increased risk with increasing maternal age included deterioration with age of the intrauterine environment, degradation of ovum between the time of differentiation and the time of ovulation, and the cumulative effect of mutagenic agents on the maternal gonad [63]. James [63] claimed, in his study of 781 women, that the correlation between increasing maternal age, increasing birth order, and an increased occurrence of abortion was due to the fact that "abortion-prone" women have more pregnancies and are older at the time of pregnancy than "average" women. Supporting the idea that abortion-prone women have more than the normal number of pregnancies are the findings of Warburton and Fraser [167] which showed that the mean interval between pregnancies was decreased significantly compared to controls (from 21 to 12 months) if the previous pregnancy ended in an abortion. In a study of 2,133 families, these authors noted that, while the risk of the first abortion increased with maternal age, once an abortion had occurred, there was no further increase in abortion risk with increasing maternal age. A more recent study [73] substantiates that gravidity itself is not a factor in spontaneous abortion, although other studies have found contradictory results [150, 167]. The finding that an increased maternal age is a significant factor in trisomic abortuses [15, 34, 80] suggests that the correlation with maternal age reported in previous studies was due to the increased risk of nondisjunction and subsequent termination of pregnancy. Early work with Drosophila and mice has indicated that an increase in nondisjunction may be associated with a decrease in crossing over, and that decreased crossing over and

terminalization of chiasmata occur more frequently with increasing maternal age [12]. While the mechanism of control of nondisjunction in humans is not clear, the same age-influenced mechanisms may be involved [54].

A variety of environmental factors have been implicated in an increased risk of chromosomal abnormalities; the genotoxic effect may occur in the oocytes, spermatocytes, or in the fetus itself. Some of the implicated genotoxic factors include ionizing radiation, infections or rubella virus, environmental chemicals, and drugs. Studies have shown that x-ray irradiation is a significant factor in increasing the risk of spontaneous abortion in humans. Alberman et al [2] compared the total ovarian dose of x-ray from routine and therapeutic irradiation in 845 mothers having spontaneous abortion to that of control mothers matched for age, race, and time of conception. Control mothers were those whose pregnancies terminated with the birth of a child. As shown in Table 4, mothers of abortuses with abnormal karyotypes were shown to have the highest mean dose of x-ray irradiation, and mothers of live-borns (controls) had the lowest mean radiation dose. This difference was statistically significant. Mothers of abortuses with normal karyotypes had a lower mean radiation dose than those with abnormal karyotypes. This difference, however, was not statistically significant. Abortions of unknown karyotype were intermediate between the normal and abnormal karyotyped abortuses. Alberman and co-workers concluded that maternal irradiation increases the risk of chromosomal damage and, consequently, the risk of spontaneous abortion. These authors felt that the data collected on paternal irradiation was not of sufficient quality to analyze.

2.2.2. Other causes of spontaneous abortion.

2.2.2.1. Immunological factors. While chromosome abnormalities and the factors that increase the risk of those abnormalities are extremely important, 40% of recognizable first-trimester abortions and an even greater proportion of second-trimester abortions are due to other factors. One of the areas of long-standing interest is the immunological aspect of pregnancy and fetal loss. In normal pregnancies, the fetus is protected from a maternal immune response [7]. This protection appears to originate in the trophoblast, which is the layer of fetal tissue in direct contact with maternal cells at the placenta. Simmons and Russell [142] have shown in mice that trophoblastic tissue does not elicit an immune response regardless of its placement, and that if an immune response is artificially induced the trophoblast is resistant to it. The lack of effect of the immune response on the trophoblast is illustrated also by the remarkably high metastatic ability of choriocarcinomas, a derivative of trophoblast cells. Of interest here is that the protective mechanism of the trophoblast may fail, and thus the fetus

TABLE 4. Mean Ovarian Dose of X-Irradiation in Millirads (From Alberman et al [2])

		Abortions			
	Controls	Normal karyotypes	Unknown karyotypes	Abnormal karyotypes	All abortions
Mean dose in millirads	180.4	204.5	252.4	339.7	224.8
N	845	303	439	105	845

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may suffer damage or loss from the maternal immune system. The induction of "runt disease," which occurs following infiltration of maternal lymphocytes, may occur in humans if either maternal blocking factors or the immune competence of the fetus are impaired [7]. It has been noted also that the greater the antigenic difference between the mother and the fetus is, the heavier will be the placenta [7].

While it is known that HLA-A and -B antibodies can be formed as a result of feto-maternal incompatibility and that these antibodies can cross the placenta, no significant correlation was found between HLA-A and HLA-B antibodies and spontaneous abortion in a study of 1,726 pregnant women [1]. However, the results of the study did show an increased level of antibodies with increasing parity. Furthermore, Burke and Johansen [19] found that antigenic stimulation was greater in a full-term pregnancy than in pregnancy ending in spontaneous abortion. It has been suggested that some mechanism exists that prevents the production of Migration Inhibitory Factor (MIF) by maternal lymphocytes in response to paternal antigens. Rocklin et al [132] showed that serum factor from normal multigravida women contained this blocking factor, but that it was dramatically lower in women who had suffered three or more spontaneous abortions. This serum factor has been found to be an IgG antibody, and while the sample sizes in these studies were small, they do indicate that the absence of these blocking factors may result in damage to the fetus or placenta.

The study of HLA compatible and incompatible matings has led Schacter and co-workers [140] to conclude that compatible matings carry a higher risk of spontaneous abortion than do incompatible matings. In 77 couples the frequency of those sharing HLA-A and -B antigens was significantly higher in 23 couples with recurrent spontaneous abortion and in 21 couples with a history of an offspring with a neural tube defect than in 17 couples with three or more normal pregnancies. The extent of HLA compatibility was also significantly higher than expected in 16 couples with only one spontaneous abortion. Schacter and co-workers [140] proposed that the HLA locus may be linked to a recessive mutant with several alleles. This type of recessive mutation, which leads to fetal death, is analogous to the murine T-complex that has been linked to the major histocompatibility locus of the mouse. Because of linkage disequilibrium, couples sharing HLA genes would be more likely to conceive a homozygous mutant. The data are conflicting since several studies have shown no deviation from the expected frequency of HLA-A and -B compatibility in matings resulting in spontaneous abortion [83].

HLA-D, which governs the Mixed Lymphocyte Culture Reaction (MLC), is thought to express the degree of cellularly recognized antigenic differences. Using an MLC of husband and wife lymphocytes, Lewis et al [90] found that there was a diminished maternal response, as measured by the percentage of transformed cells, in the MLC from cultures of cells from pregnant compared to nonpregnant couples. When classified by gravida status, decreases were found in the percentage of cell transformations and in the ratio of female-to-male cells transformed if the mother was multigravida. This indicated a lack of responsiveness by the female cells. Jenkins and Hancock [64] confirmed this observation using two designs. First, maternal cells and fetal umbilical cord blood cells from 20 primigravida and 25 multigravida women were compared to primigravida women (Table 5). The second design used maternal cells from late pregnancy and paternal cells, and, again, multigravida cases had a significantly lower reponse than did primigravida cases (Table 5). The relationship of decreased maternal response and fetal loss has been examined by Halbrecht and Komlos [52] in a study in

which 22 women with one to three spontaneous abortions and six women with hydatidiform moles were compared to a control group of fertile couples and infertile couples with a known organic cause for infertility. As shown in Table 6, an increased risk for spontaneous abortion appears to be associated with a higher MLC response. The control group had only a 5.7% cell transformation, whereas the aborters were characterized by cell transformation rates of 14.0%, 17.1%, and 19.8% for one, two, and three abortions, respectively. Those couples with a hydatidiform mole had the highest response (21.6%). Ohama and Kadotani [119] obtained similar results in an examination of couples who were either spontaneous aborters or infertile for unknown reasons (Table 7). The infertile group was characterized by a significantly higher MLC response than either fertile couples or random, unrelated couples. The MLC response in the couples with spontaneous abortions was similar to that seen in infertile couples. However, in another study, Lauritsen et al [84] using a one-way MLC assay, found that

TABLE 5. MLC Response in Maternal, Paternal, and Fetal Cells in Women With Different Reproductive Histories (Jenkins and Hancock [64])

	N	Parity	% Mean transformation
Maternal and fetal cells	20	0	13.79*
	25	+1	7.80*
Maternal and paternal cells	6	0	13.00**
	7	+1[a]	3.64**

*Difference in means significant at $P<0.025$.
**Difference in means significant at $P<0.02$.
[a]Maternal cells from late pregnancy.

TABLE 6. Maternal and Paternal Cells MLC in Women With Spontaneous Abortions, Hydatidiform Moles, and Controls (Halbrecht and Komlos [52])

Group	N	% Transformation	(Range)
One spontaneous abortion	8	14.0	(5–33)
Two spontaneous abortions	9	17.1	(12–21)
Three spontaneous abortions	5	19.8	(15–33)
Hydatidiform mole	6	21.6	(6–35)
Controls	8	5.7	(2–8)

TABLE 7. Maternal and Paternal Cells MLC in Couples With Various Reproductive Histories (Ohama and Kadotani [119])

Group	N	% Transformation
Sterile	16	10.1 ± 6.8
Abortions	18	9.2 ± 6.5
Fertile	13	4.7 ± 6.5
Unrelated	101	6.6 ± 5.5

MLC reactivity of maternal cells to the husband's cells was decreased in women with recurrent spontaneous abortion.

For many years, ABO incompatibility has been suspected of being a factor in the etiology of spontaneous abortion, and many studies showing a distortion of blood type distributions in offspring or a deficiency of live-born children in ABO incompatible matings have been reported [88]. Rh incompatibility also is known to be of great importance in the survival of an infant; it is the underlying pathology of erythroblastosis fetalis. The largest and most comprehensive studies of the effect of ABO and Rh incompatibility on pregnancy outcome are those of Cohen [27, 28], in which the outcome of pregnancies for 7,770 Caucasian matings of known ABO and Rh type were recorded. The effect of incompatible matings (defined as a mating in which the mother lacks an antigen that her husband possesses) was calculated in several ways, as shown in Table 8. When the study sample was classified according to early and late fetal death, and the total fetal death index (fdi) was calculated, it was found that the fdi was 26% higher in simple ABO incompatible matings than in ABO compatible matings. Simple ABO incompatible matings resulted in early spontaneous abortions, whereas the loss for Rh incompatible mating was distributed over all gestational ages. In this study, no distortion of the distribution of blood type was seen in the offspring, and Cohen theorized that selection against the overall "biological fitness" of the mating was occurring rather than selection against the blood type of the fetus itself.

In 1943, Levine [89] reported a deficiency of ABO incompatible matings among mothers of infants with Rh erythroblastosis fetalis. Since then, the protective effect of doubly incompatible matings has been well established. Cohen [28] found the same effect, and posed the question of which locus was providing the protection. She reasoned that if the fdi for doubly incompatible matings was lower than the fdi for simple Rh incompatible matings, then ABO incompatibility afforded a protective effect against Rh selection and vice versa. Cohen found protection present in both directions, although ABO protection appeared to be stronger.

Convincing evidence of the role of ABO incompatibility comes from a study by Takano and Miller [154] of 229 maternal and abortus blood types. The distribution of maternal blood types was not found to differ from that reported for a normal population; however, the total number of incompatible abortuses observed was significantly greater than expected in the 78 cases examined. Furthermore, there was no apparent interaction between ABO and Rh incompatibilities. Although no systematic chromo-

TABLE 8. Methods of Calculating the Effect of Incompatible Matings (Cohen [27])

$$\text{Total fetal death index (fdi)} = \frac{\text{fetal deaths (all gestational ages)} \times 100}{\text{live births}}$$

$$\text{Early fetal death index} = \frac{\text{early fetal death (20 weeks gestational age)} \times 100}{\text{live births}}$$

$$\text{Late fetal death index} = \frac{\text{late fetal deaths (+ 20 weeks gestational age)} \times 100}{\text{live births}}$$

$$\text{Neonatal death index} = \frac{\text{neonatal deaths (28 days after birth)} \times 100}{\text{live births}}$$

$$\text{Indicator ratio: } \frac{\text{specific death index for a given incompatible ABO and/or Rh mating}}{\text{specific death index for a given compatible ABO and/or Rh mating}}$$

some analysis of the abortuses was performed, the authors did note a higher frequency of physical abnormalities in the compatible group. They felt that this finding might be consistent with the existence of chromosome abnormalities. Studying the causes of spontaneous abortion, Lauritsen et al [80] compared blood type frequencies in chromosomally normal and abnormal groups. In 288 cases of spontaneous abortion, ABO incompatible matings were found in 44% of the karyotypically abnormal group. Among the maternal-fetal incompatible pregnancies, a significantly higher-than-expected frequency of abortuses with normal karyotypes was seen.

The existence of antibodies to sperm in both males and females has been recognized for nearly a century, but the relationship between this immunologic effect and fertility was not recognized until 1954 when Wilson [121] discovered sperm agglutinin in human semen and blood. Franklin and Dukes [43] reported sperm agglutinin activity in 78% of women with unexplained infertility as compared with 11.8% of women with known fertility and 10.37% of women with a known organic cause for infertility. Jones [68] conducted sperm agglutinin and sperm immobilization tests on a total of 427 women, and found sperm agglutinin activity in 26.5% of the cases of unexplained infertility as opposed to sperm agglutinin activity in 18% of pregnant controls. Serum from women with essential infertility and from women with minor organic problems caused a higher degree of sperm immobilization than was seen with serum from pregnant controls. The rate of spontaneous abortion in the women with sperm agglutinin antibodies was 54% as compared with a spontaneous abortion rate of 20% in those without the antibody.

2.2.2.2. Infections. Viruses, bacteria, and parasites are suspected of causing spontaneous abortions through either infection of the mother, infection of the fetus, or damage to the chromosomes. It has been very difficult to specify the types of infections that are endangering, and the degree of danger that they present, particularly in the case of viral infections. There is clear evidence that bacterial infections of the endometrium can cause abortion or can lead to the development of endometrial adhesions [151]. Endometrial infections by staphylococcus, streptococcus, E. coli, E. intermedia, Neisseria gonorrhea, and pseudomonas have all been implicated in causing spontaneous abortion [151]. Infection of the fetus, particularly by the gram-negative bacilli such as hemolytic streptococcus and Staphylococcus aureus, can lead to meningitis, encephalitis, and other central nervous system pathology. Fetal mortality is high in these cases, but the occurrence of such infections appears to be quite low [5]. Untreated maternal infections of syphilis increase the risk of spontaneous abortion up to 30% [5].

In animal studies, infections of Listeria monocytogenes have increased the incidence of abortions [160]. Although transplacental infections of Listeria have occurred in human fetuses [160], in a study of 554 human abortions Tabau and David (1963) found no evidence of Listeria infection, indicating that its occurrence may be very low. Chlamydia trachomatis, a common venereal pathogen, has been implicated as a causal agent of salpingitis. Tubal occlusion following salpingitis is a major cause of sterility in women; however, there is no evidence that establishes a relationship between C. trachomatis and early fetal loss [100].

Mycoplasmas are found in the genital tract of 40–70% of healthy adults [151], and certain strains are known to cause fetal wastage in mice. In humans, Sompokinsky et al [146] found a greater incidence of mycoplasma infections in spontaneous abortion

(both early and late abortions) than in induced abortions. However, it is possible that the mycoplasma infections were a consequence of fetal death and not causal factor in abortion. Stray-Pederson [153] recently reported a higher incidence of T-mycoplasma infections of the endometrial tissues among 16 patients with a history of habitual abortion (28% occurrence rate) when compared with 45 control patients (7% occurrence rate). Among 18 women with primary infertility, 50% had T-mycoplasma of the endometrium.

Maternal infection with the protozoal parasite toxoplasma gondii can be transmitted to the fetus and cause extensive damage [72]; however, a cause-and-effect relationship between maternal toxoplasmosis and spontaneous abortion has not been demonstrated. Results of a prospective study of 5,000 obstetrical patients by Kimball et al [72] indicated that habitual abortion and toxoplasmosis were not causally related. While chronic toxoplasmosis may increase the risk of what Kimball et al [72] called "sporadic abortion," the occurrence of such cases was low. Acute infections did not appear to increase the risk of abortion.

Many studies indicate that viruses are teratogens, the most well-known manifestation of such being the rubella syndrome. Although viral infections may lead to fetal malformations, the relationship between viral infections and spontaneous abortion is not well defined. In congenital rubella infections, increased fetal mortality has not been clearly demonstrated; the majority of mortalities apparently occur postnatally [77, 141]. Cytomegalovirus is the virus most commonly seen in pre- and perinatal infections, but its effect on fetal mortality is not known [79]. Berenberg and Nankervis [9] noted that women who gave birth to infants with clinical cytomegalic inclusion disease tended to have spontaneous abortions in the next pregnancy. But another study did not substantiate their findings [78]. Kriel and co-workers [75] found that about 10% of aborted fetuses were positive for cytomegalovirus infection. Viral particles resembling cytomegalovirus were found in an additional 15% of abortuses. These findings were not confirmed by Boué and Loffredo [13]. Genital herpes simplex presents a substantial risk to the newborn if the mother has an active infection at the time of delivery. Also, there are data suggesting an increased risk of spontaneous abortion due to genital herpes simplex infection. Naib and co-workers [114] found a 33.3% abortion rate in pregnancies in women with a positive genital herpes test, compared to a 9.4% rate in women with a negative herpes test.

Varicella-zoster, mumps, and coxsackie A and B infections have not been shown to increase the risk of fetal loss [30, 141]; however, maternal infection of variola-vaccinia virus (small-pox) results in either a marked increase in fetal death if the infection occurs in the first half of gestation or a premature delivery if it occurs in the second half of gestation [95].

Influenza infection is believed to increase the risk of spontaneous abortion, although it has not been clearly established. Krugman [77] indicated that the risk of abortion is dependent on the severity of maternal infection [77], although fever or drug therapy during the course of infection may be the cause of fetal loss rather than the virus itself [98]. Maternal infection with poliomyelitis virus during the first trimester has been found to result in spontaneous abortion during the 2-week period following the onset of polio symptoms [160].

2.2.2.3. Endocrine factors. Luteal phase defects are important factors in infertility and spontaneous abortion [8]. The diagnostic criteria for these defects include a luteal

phase of less than 10 days' length as calculated from changes in basal body temperatures, changes in serial measurements of pregnandiol during the luteal phase, and histologic evidence that endometrial development lags behind basal body temperature by more than 2 days [8]. Luteal phase defects may be caused by a reduced production of gonadotropin, which affects both FSH and LH, or they may be caused by pathologic changes in the ovaries or corpus luteum. The net effect of these malfunctions is a decrease in the level of progesterone, a hormone necessary for maintaining a pregnancy. Based on the criteria listed above, luteal phase defects occurred in 11.5% of 335 women studied [8]. However, only 3.9% of these women had a repeated cycle of luteal phase inadequacy. Since luteal phase defects would only be detected clinically if they were repetitive, it is unlikely that they are a major cause of recognizable spontaneous abortion. Hernandez-Horta et al [58] compared levels of plasma progesterone during the luteal phase in habitual aborters (more than three abortions) and healthy nonpregnant and pregnant controls, and found that habitual aborters had lower progesterone levels during the luteal phase than did nonpregnant controls. Furthermore, using data from the normal pregnant controls, the authors calculated normal plasma concentration of progesterone for the 1st–6th and 5th–12th weeks of pregnancy (with means of 13.6 ng/ml $\pm$ 0.46 and 22.8 ng/ml $\pm$ 0.80, respectively). When several women in the habitual aborter group became pregnant, their progesterone levels were lower than the control average level. All of the habitual aborters who became pregnant aborted between the 7th and 12th week of gestation, and their progesterone levels were less than 6 ng/ml at 48–72 hours prior to bleeding or abortion.

The ability to maintain a pregnancy depends not only on those hormones directly related to pregnancy, but also on hormones necessary for the normal endocrine function of the mother. Defects or malfunctions of the thyroid are known to alter basal metabolism and to influence the ratio of estrogen to progesterone [20]. Studies on hypothyroid animals have shown that, while their fertility does not seem to be impaired, their ability to maintain pregnancy is decreased [74]. The hypothyroid woman has been shown to have a significantly higher incidence of premature births and of stillbirths, an increased risk of spontaneous abortion, and an increased risk of children with malformations and mental retardation [50, 67]. Hyperthyroidism, having a frequency rate as high as 2 out of every 1,000 pregnancies [118], is more commonly seen in pregnancy than hypothyroidism. Thyrotoxicosis has been associated with a slight increase in neonatal mortality, and with a significant increase in low birthweight babies. In thyrotoxicosis, there does not appear to be a significant risk of spontaneous abortion [161], but Talbert et al [155] have reported a spontaneous abortion rate of 11.5% in hyperthyroid women being treated with drugs and a rate of only 3.5% in a group that was surgically treated.

Pregnant women with untreated hypoparathyroidism may have offspring with neonatal hyperparathyroidism, but, if the mother's parathyroid disease is medically controlled, there appears to be no deleterious effects in the offspring [17]. In women with hyperparathyroidism, Johnstone et al [65] found five spontaneous abortions and five stillbirths among 44 pregnancies. Hyperparathyroidism in the mother, a condition that causes the fetus to be exposed to an abnormally high level of calcium, also results in low birthweight babies.

Pregnancy can exacerbate conditions such as Cushing syndrome, causing an increase in the number of stillbirths and prematurities [46]. Adrenal insufficiency (Addison disease) does not appear to increase the risk of spontaneous abortion.

Pregnancies in diabetic women present a special problem to the obstetrician, and much has been written concerning the association of diabetes and the increased number of stillbirths, neonatal deaths, and congenital malformations [123]. It is not clear whether diabetes is also associated with an increased risk of spontaneous abortion. In a study by Drury et al [37], there were 60 spontaneous abortions out of 618 pregnancies in diabetic women; this occurrence was similar to the average rate in controls.

2.2.2.4. Physical abnormalities. Physical abnormalities of the uterus and cervix do not seem to cause a decrease in fertility, but do appear to decrease success in maintaining pregnancy. Population studies have revealed that approximately 3% of all women have a malformation of the reproductive tract, the most common being subseptate uterus and bicornate uterus [33, 93]. According to Fenton and Singh [41], the incidence of spontaneous abortion is between 24% and 53% in pregnancies of women with a malformation of the reproductive tract. Craig [33] reported that, of 57 women with reproductive tract malformations, nearly half had recurrent mid-trimester spontaneous abortion. Some physical malformations of the reproductive tract have been shown to have a genetic component. Uterine abnormalities are associated with several genetic syndromes such as Fraser syndrome, hand-foot-uterus syndrome, and Meckels syndrome [143]. Abortion is also frequently seen in women with fibroid uterus due to leiomyomas [33]. Racial differences in the incidence of leiomyomas, along with the observation that leiomyomas were more common in relatives of affected than of control individuals, raise the possibility that genetic factors play a role in this cause of second-trimester abortions [170].

2.2.2.5. Environmental and occupational hazards. The relationship between smoking during pregnancy and small-for-date babies has been fairly well established. In addition to an effect of maternal smoking on term pregnancies, some studies have shown an increase in "unsuccessful" pregnancies [136]. These same workers demonstrated a significant increase in stillbirths, neonatal deaths, and abortions among 712 smokers as compared to 1,545 nonsmokers (Table 9). Many studies have shown a wide variation in the magnitude of risk involved, but generally it is agreed that smoking increases the risk of fetal and perinatal death [105]. Recently, Warburton and co-workers [168] have defined more clearly the risk of spontaneous abortion in smoking women. As shown in Table 10, the study included 414 cases of spontaneous abortion and 428 controls. Nearly 42% of the women with spontaneous abortion reported that they smoked during the pregnancy compared to 28% of the control women. Warburton and co-workers also found that alcohol consumption was more frequent among the mothers of spontaneous abortions than among controls (Table 11). In view of the sus-

TABLE 9. Effect of Smoking on the Outcome of Pregnancy (Russell et al [136])

Outcome	Nonsmokers	Smokers
Unsuccessful	66	54
Successful	1,479	658
Total	1,545	712

$\chi^2 = 10.62$, df = 1; $P < 0.01$.

TABLE 10. Effect of Smoking on Spontaneous Abortion (Warburton et al [168])

Smoking status	% Cases	% Controls
Nonsmoker	58.2	72.0
Smoker	41.8	28.0
Total number	414	429

$\chi^2 = 11.64$, df = 1; $P < 0.01$.

TABLE 11. Effect of Alcohol Consumption on Spontaneous Abortion (Warburton et al [168])

Alcohol consumption during pregnancy	% Cases	% Controls
Never or rarely	62.7	76.4
Monthly	18.2	16.6
Weekly	13.6	5.8
Daily	5.5	1.2
Total number	413	428

$\chi^2 = 24.41$, df = 3; $P < 0.01$.

pected teratogenic effect of alcohol on the fetus, it is logical that alcohol may cause developmental damage that would lead to spontaneous abortion.

The effect of occupational exposures on the incidence of spontaneous abortion is of increasing concern in highly industrialized countries. A general trend has been reported that a higher infant mortality rate exists among working women [166] than among nonworking women; however, the specific agents involved have been difficult to identify. Exposure to metals such as mercury, cadmium, and lead have been shown to increase the risk of spontaneous abortion [16]. In a questionnaire study of nurses and nurse anesthesiologists, Cohen and Bruce [29] found a higher incidence of spontaneous abortions and congenital malformations in pregnancies of women exposed to anesthetic gases than of an unexposed population. Exposure to various pesticides, particularly the polycylic hydrocarbons, have also been implicated in causing an increased risk of spontaneous abortion [166].

An increase in fetal loss was seen in the wives of men who had been exposed to vinyl chloride monomer (VCM); no such increase was seen in the same study after exposure to the polymer, polyvinyl chloride (PVC) [60]. Chloroprene (2-chlorobutadiene), which is chemically related to VCM, was found to cause a threefold increase in the spontaneous abortion rate in the wives of exposed workers [137]. Also, Sanotskii [137] reported morphologic disturbances of spermatogenesis in exposed workers.

2.2.2.6. Trauma. Trauma seems to have little effect on the fetus during the first trimester since it is well protected by the uterus and the pelvis itself [21]; however, direct trauma can cause death of the fetus. Burns can be a major factor in spontaneous abortion. If over 35% of the body surface of the mother is burned severely, premature labor is highly probable [147].

2.3. Recurrence Risks and Habitual Aborters

The probability of a recurrence of spontaneous abortion is a critical clinical and experimental question. Studies done without the benefit of chromosome analysis have shown that the risk for recurrence is dependent on the previous pregnancy history. Leridon [85] collected data on 3,185 pregnancies and found that, after a spontaneous abortion during the first pregnancy, the risk of abortion during the second was 28%. If abortions occurred during the first two pregnancies, the abortion risk for the third was increased to 38%, and to 50% in the fourth pregnancy if the first three pregnancies were aborted. Women without a history of spontaneous abortion in the first pregnancy had a risk of 16% for the second pregnancy and 17.5% and 12.6% for the third and fourth pregnancies, respectively. Poland and co-workers [126] have also investigated the recurrence risk by following 1,118 women who had a spontaneous abortion. Their results support those of Leridon in that a history of fetal loss increased the risk of subsequent fetal loss. The presence of a malformation in the aborted fetus was found to increase further the risk of a subsequent abortion. Further clarification of the association between the existence of a congenital malformation in the abortus and increased risk of subsequent abortions has been provided by Lauritsen [80] who found that, if the mother had had a spontaneous abortion prior to the occurrence of a subsequent abortion, the frequency was 33% regardless of a prior history of live-born children. Women with no previous history of spontaneous abortion had a subsequent abortion frequency of 15%. Lauritsen [80] also found a significant difference between karyotypically normal and abnormal index cases in the rate of spontaneous abortion. The frequency of occurrence of a subsequent abortion when the index case was chromosomally normal was 26% compared to 14% when the index case was chromosomally abnormal.

The subgroup of women or couples with habitual abortions, usually defined as two, three, or more spontaneous abortions, has been studied by several investigators. There is some dispute as to whether or not the frequency of translocation carriers is higher among the parents of aborted fetuses compared to the normal population. Among 18 women with two or more spontaneous abortions, Kajii and Ferrier [70] found three balanced translocations and one with a 47/XXX constitution. Neu et al [116] found only one female with t(13q14q) out of 30 couples with three or more spontaneous abortions. Ward and co-workers [169] found no translocation carriers among 100 couples with two or more abortions, although they did find six chromosomal variants such as pericentric inversion or prominent satellites and one female with 45X/46XX mosaicism. Other studies have shown that 3–31% of couples with habitual abortions are translocation heterozygotes [22, 149, 158].

Many investigators have reported an increase in the frequency of occurrence of a large Y in couples with habitual abortions compared to controls [45, 117, 122]. Genest [45] studied 51 couples with two or more spontaneous abortions, and found that all of the females had normal karyotypes, but nearly 23% of the males had a Y chromosome variant or abnormality: Ten of the males had Yq^+, one had Yqs, and one had two Y chromosomes.

The various causes of habitual abortion have been summarized by Tho et al [158] in their study of 100 couples with two or more abortions or with one abortion and a history of a phenotypically abnormal child. As shown in Table 12, 25% of couples

TABLE 12. Causes of Spontaneous Abortion in 100 Couples (Tho et al [158])

Etiology	N	Comments
"Genetic"	25	12 Balanced translocations 13 "Multifactorial"—previous history of offspring with: neural tube defects (8), Potter syndrome (2), diaphragmatic hernia (1), omphalocele (1), cleft lip and palate (1)
"Mullerian"	15	7 Uterus subseptus 3 Uterus septus 1 Fibroid uterus 4 Cervical incompetence
"Endocrine"	23	All with endometrial asynchrony or retarded endometrial development
"Unknown"	37	

had a "genetic etiology" that increased the abortion risk. About half of these were translocation heterozygotes, and half were "multifactorial" causes. Fifteen percent of all couples were defined as having "Mullerian" problems, either malformations of the uterus or cervical incompetence. Twenty-three percent of the couples had endometrial asynchrony or retarded endometrial development (luteal phase defects) due to problems in endocrine function. Finally, in 37 couples, no cause for the repeated abortions could be identified. As techniques and knowledge increase, more causes of spontaneous abortion may be defined.

3. MATERIALS AND METHODS

3.1. Case Ascertainment From the Norwegian Twin Registry

The Norwegian Twin Registry (NTR) was initiated in 1954 by Drs. Odegard, Mohr, and Kringlen to serve as a base population from which participants could be recruited for a series of psychiatric studies [76]. Until recently the registry consisted of index cards with the names, birthdate(s), parents' names and birthdates, and the residence of the mother at the time of the twins' birth for all twins born since 1895 who were registered by birth certificate. The registry is currently under the supervision of Drs. Berg and Kringlen at the Institutes of Medical Genetics and of Psychiatry, respectively. Updating of name changes and addresses, in addition to computerization of the registry, was initiated in the fall of 1978.

The process of updating the NTR is diagrammed in Figure 2. Updating of information on opposite-sexed twins was postponed until the updating of same-sexed twins was complete. Since the particular variables of interest involved adult twins, pairs of whom one or both twins were stillborn or died early, or where both members had emigrated, were set aside. The remaining same-sexed twin pairs were linked with the Norwegian Central Statistics population file to obtain current addresses and names. The population file, updated July 1978, contains the name, address, and personal number (equivalent to Social Security number) of all individuals born in Norway and still alive as of 1964. Information on emigration and deaths occurring after 1964 is also included. There is limited access to this file for confidentiality purposes, and permission to use it for this study was granted by the Norwegian Central Statistics Bureau.

Twin pairs for whom a current address was found for one or both members were computerized using the first of three major data entry programs, NEWTWIN. For those twin pairs for whom an address could not be found for either member, the most likely local registry, based on the mother's place of residence, was

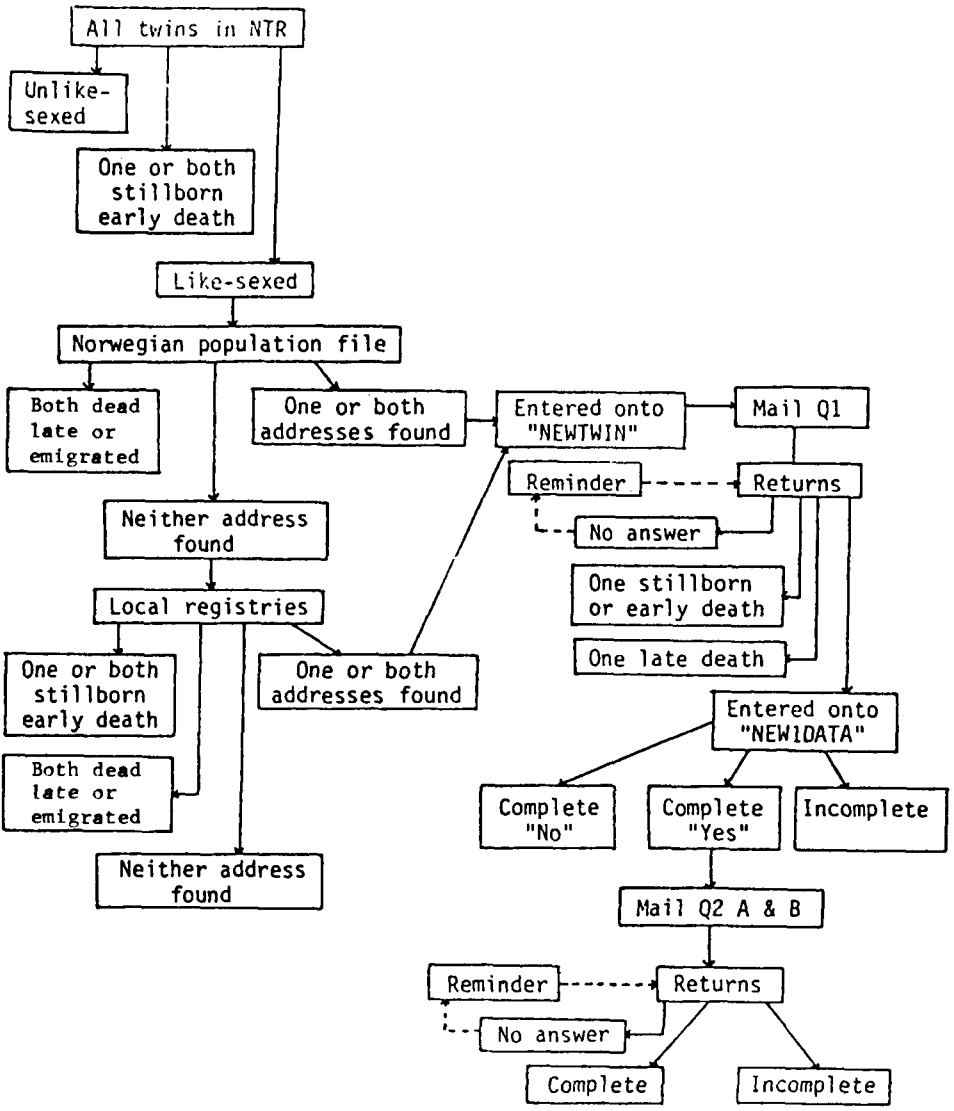

Fig. 2. Flow diagram of Norwegian Twin Registry updating.

identified, and a search of the files was requested. The full cooperation of the local registries throughout Norway enabled the collection of more complete information concerning stillbirths and early death of twins. A significant number of addresses of living twins were also obtained from this secondary source, and these were also passed to the twin registry computer file.

Twins included in the NEWTWIN file were sent a questionnaire packet consisting of an introductory letter and a zygosity questionnaire, Q1 (Appendix 5). Twins who were not interested in participating in the registry or who were found to have a stillborn cotwin or a cotwin who died early or after the age of 20 were not included in the NEW1DATA file. The twins contacted were divided into three categories: 1) pairs of whom both twins returned the questionnaire but one or both responded "no" to the question of receiving further questionnaires ("Complete No"); 2) pairs of whom only one twin returned the questionnaire ("Incomplete"); and 3) pairs of whom both twins returned Q1 and agreed to receive further questionnaires ("Com-

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plete Yes"). If an answer had not been received after approximately 4–5 weeks, a reminder letter and new questionnaire were sent.

A second questionnaire packet consisting of an explanatory letter, two general health questionnaires (Q2A), and a reproductive history questionnaire (Q2B) were sent to the "Complete Yes" twins. Responses from Q2A and B were computerized and entered into the NEW2DATA file. Some of the data were abstracted by hand. The questionnaires used in this study were designed by the Department of Human Genetics at the Medical College of Virginia and the Institute of Medical Genetics at the University of Oslo. Translation into Norwegian was done by the Institute of Medical Genetics. Appendix 5 shows a sample of the English version. The Norwegian versions are essentially identical both in form and content except that the zygosity question was modified to read "were you as alike as two drops of water" instead of the usual "were you as alike as two peas in a pod." The data entry programs, NEWTWIN, NEW1DATA, and NEW2DATA, were designed for this project by Knut Smaaland and Egil Pedersen of the University of Oslo Computer Center.

The zygosity questionnaire, Q1, was tested for its ability to discriminate MZ and DZ twins by conducting a blood typing study on 208 pairs of twins within a 2-hour driving radius from Oslo. The area covered contains about 40% of the total Norwegian population. Thirteen reliable markers were used in the initial evaluation although others may be included at a later date (Magnus, in preparation). The 13 markers studied included ABO, Rh, MNS, Duffy, Kidd, Kell, C3, Lp(a), haptoglobin, P, Lewis, Ag(x), and Lutheran. Details of the collection, laboratory analysis, and computer analysis are given elsewhere (Magnus, in preparation). Questionnaire evaluation included the following process: If the twins responded that they had "always" been mistaken for each other by their grandparents, they were classified as MZ. No DZ twins answered this question "always." The remaining twins were tested for the responses to the hair color question. Those responding that their hair color was "unlike" were classified as DZ. No MZ twins responded that their hair color was unlike. The twins still remaining after these two tests were subjected to a stepwise discriminant analysis using a BMDP packet and using questions 4, 5a–f, and 6a–l. Question 4 is the "two drops of water" question. Questions 5a–f concern the ability of relatives, teachers, and strangers to tell the twins apart, and in questions 6a–l the twins were asked to note how alike they thought they were to each other.

The following data were abstracted from Q1 and Q2 A and B for the spontaneous abortion study:

1) Family number	A six-digit number assigned sequentially to half-sib families in this study. Digits coded as: first two, year of birth of twins; next three digits, the sequential number within the birth year; last digit indicates first or second twin.
2) Birthdate of twin	
3) Birthdate of spouse	
4) Sex of twin	
5) Pregnancy results	The sex, order, and number of the outcome of pregnancy designated by an alphabetical code A–L.
6) Height of twin	
7) Height of spouse	
8) Age of menarche	For female twins and the spouses of male twins. Fractions rounded.
9) Age of menopause	For female twins and the spouses of male twins. Fractions rounded.
10) Date of first pregnancy	Given as the date of the first birth.
11) Year of marriage	Only the year of marriage was requested.
12) Zygosity	

3.2. Statistical Methods

3.2.1. Analysis of the maternal effect in first-trimester spontaneous abortion. The qualitative half-sib model, which is an extension of the quantitative half-sib model described elsewhere [115], was used to analyze for maternal effects on the incidence of first-trimester spontaneous abortions. Under an assumption of complete selection through the twin parents, it is assumed that a given

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parent is either genetically at risk (R) with probability p or not at risk (N) with probability (1-p) for having a spontaneous abortion. The probability that a pregnancy will be affected depends on the combination of parental risk; for a given half-sibship there are eight possible combinations of parental types, each associated with a specific risk to the offspring of twin 1 or twin 2 (G_i1 and G_i2, respectively) as is illustrated in Table 13 for maternal half-sibships. The probability of a given half-sibship can thus be expressed in terms of five parameters: p, the probability that a parent is at high risk; a, the probability of an affected offspring is the mother is at high risk; b, the probability of an affected offspring if the father is at high risk; c, the probability if both parents are at high risk; and w, the sporadic risk assumed to be present regardless of the status of the parents. The probability in terms of the frequency of the parental mating types, f_i, the risk factors G_i1 and G_i2, the number of affected, r, and total, s, pregnancies can be expressed as:

$$P_{r_1 s_1 r_2 s_2} = \binom{s_1}{r_1}\binom{s_2}{r_2} \sum_{i=1}^{8} (f_i)\,(G_i1)^{r_1}\,(1 - G_i1)^{s_1 - r_1}\,(G_i2)^{r_2}\,(1 - G_i2)^{s_2 - r_2}$$

The log of this function summed over all half-sibships represents a log likelihood function for the sample from which estimates of the parameters can be obtained. Each parameter can be estimated separately, or dependence on other parameters can be specified. A generalized maximum likelihood routine, MAXLIK, developed by Kaplan and Elston [71], was used to obtain parameter estimates.

MAXLIK operates using two user-supplied subroutines that allow the user to designate dependent variables (DEPAR) and to estimate a log likelihood under a proposed model (LOGLIK). The program designed for the analysis of MZ half-sib data included three additional subroutines called by LOGLIK. The iteration procedure chosen included a direct search of the likelihood surface and one iteration of the Newton-Raphson method.

TABLE 13. Calculation of Expected Risks in Maternal Half-Sibships

Parental mating type □—○—□	Frequency of parental combination (f_i)	Female twins: 1st sib risk G_i1	Female twins: 2nd sib risk G_i2
(1) R R R	p^3	w + c − cw	w + c − cw
(2) R R N	$p^2 (1 - p)$	w + c − cw	w + a − aw
(3) N R R	$p^2 (1 - p)$	w + a − aw	w + c − cw
(4) N R N	$p (1 - p)^2$	w + a − aw	w + a − aw
(5) R N R	$p^2 (1 - p)$	w + b − bw	w + b − bw
(6) R N N	$p (1 - p)^2$	w + b − bw	w
(7) N N R	$p (1 - p)^2$	w	w + b − bw
(8) N N N	$(1 - p)^3$	w	w

Where: p = probability that a parent has a "high risk"for producing an abnormal offspring (R)
(1 − p) = probability that a parent is not "high risk" (N)
a = segregation probability if mother is R
b = segregation probability if father is R
c = segregation probability if both parents are R
w = probability of a sporadic occurrence

If: r_1 = number of affected offspring in sibship 1
r_2 = number of affected offspring in sibship 2
s_1 = number of offspring in sibship 1
s_2 = number of offspring in sibship 2

Then:

$$Pr_1 s_1 r_2 s_2 = \binom{s_1}{r_1}\binom{s_2}{r_2} \sum_{i=1}^{8} (f_i)\,(G_i1)^{r_1}\,(1 - G_i1)^{s_1 - r_1}\,(G_i2)^{r_2}\,(1 - G_i2)^{s_2 - r_2}$$

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3.2.2. Analysis of other reproductive variables. Differences in the mean of male and female twins (or the female spouses of male twins when applicable) and of MZ and DZ twins for the age of menarche and menopause, the ages at marriage and at first birth, and the mean interval between marriage and the first birth were tested by the TTEST subrouting available in the Statistical Analysis System (SAS).

The mean differences between cotwins, spouses, and twins and spouses were compared for male and female twins (or the female spouses of male twins when applicable) and for MZ and DZ twins by the same SAS subroutine. Spearman correlation coefficients were obtained by another subroutine for all variables and for the total number of pregnancies, unaffected pregnancies, and first-trimester spontaneous abortions. The population of twins was divided into a "younger" group ($\leq$ 44 years of age) and an "older" group ($>$ 44 years of age) based on the present age of the twin. Differences in the age of menarche and menopause, the ages of marriage and first birth, and the interval between marriage and first birth were examined. The total number of pregnancies, unaffected pregnancies, and the number of first-trimester spontaneous abortion were also examined. The population was also divided into two groups based on the presence or absence of a reported spontaneous abortion in the pregnancy history, and the same variables were examined, excluding the number of first-trimester abortions.

A weighted least-squares procedures was used to estimate the genetic and environmental components for the age of menarche and the age of menopause. Analysis of variance and nested analysis of variance techniques were used to estimate the among-, between-, and within-mean squares of MZ and DZ twins. The genetic and environmental expectations of the mean squares are shown in Table 14. Four components were included in the analysis of the pooled age groups: V_A, the additive genetic variance; V_D, the dominance genetic variance; V_{EW}, the within-pair environmental variance; and V_{EP}, the among-pair environmental variance. The expectations of the variance components are shown in Table 15. A second analysis was used to take secular differences into account. An additional environmental variance was included, V_{ES}, which is the environmental variance among age groups. The expectations for the mean squares for twins stratified by age group are shown in Table 14, and the expectations for the variance components are shown in Table 15.

4. RESULTS

4.1. Case Ascertainment From the Norwegian Twin Registry

A total of 22,354 twin pairs born between 1915 and 1946 were contained in the Norwegian Twin Registry (NTR). These included 8,169 opposite-sexed twin pairs and 14,185 same-sexed twin pairs. Table 16 summarizes the details of the registry findings. A breakdown of registry statistics by individual year is given in Appendix 1. Appendix 2 shows the trend of live births and stillbirths recorded in the Central Statistics Archives, and Appendix 3 shows the trend of twins reported for the years 1915–1946 except for 1927–1930. Appendix 4 compares the number of same-sexed twins in the NTR with the number reported by Central Statistics. On the average, 91% of the twins reported in the Central Statistics Archives were included in the NTR. Fifty-two percent of the same-sexed pairs were females. In all, 2,024 pairs were not traceable; that is, neither twin could be found in either the population file or the local registries. One or both twins were noted to have been stillborn on the original NTR card in 1,504 pairs. Early death of one or both of the twins was noted on the NTR card in 1,753 pairs. Four hundred four pairs had been used in a previous study and were not recontacted. Thus there was a total of 8,500 same-sexed pairs for whom an address was found for one or both twins.

The first questionnaire packet, Q1, was sent to the 8,500 pairs for whom a current address was known for at least one of the pair. Table 16 summarizes the return results. In all, 2,365 complete pairs responded "yes" to the question regarding a willingness to receive further questionnaires. Incomplete pairs, of whom one twin responded and the

TABLE 14. Expected Values of Mean Squares for MZ and DZ Twins

Source of variation	Mean square (MS)	df	Expected value of MS E(MS)
Pooled age groups			
Among pairs	MS_B	$N-1$	$\sigma^2_W + k_1 \sigma^2$
Within pairs	MS_W	N	σ^2_W
Stratified age groups			
Among age groups	MS_A	$N_{AG}-1$	$\sigma^2_W + k_1 \sigma^2_B + k_3 \sigma^2_A$
Between pairs within age groups	MS_B	$N-N_{AG}$	$\sigma^2_W + k_1 \sigma^2_B$
Within pairs	MS_W	N	σ^2_W

TABLE 15. Model for the Analysis of the Age of Menarche

Source of variation	Variance component	df	Expected value of variance component
Model using pooled ages			
MZ twins			
Among pairs	$\sigma^2_{A_{MZ}}$	$N_{MZ}-1$	$V_A + V_D + V_{EP}$
Within pairs	$\sigma^2_{W_{MZ}}$	N_{MZ}	V_{EW}
DZ twins			
Among pairs	$\sigma^2_{A_{DZ}}$	$N_{DZ}-1$	$\frac{1}{2}V_A + \frac{1}{4}V_D + V_{EP}$
Within pairs	$\sigma^2_{W_{DZ}}$	N_{DZ}	$\frac{1}{2}V_A + \frac{3}{4}V_D + V_{EW}$
Model stratifying for age			
MZ twins			
Among age groups	$\sigma^2_{A_{MZ}}$	$N_{AG}-1$	V_{ES}
Between pairs within age groups	$\sigma^2_{B_{MZ}}$	$N_{MZ}-N_{AG}$	$V_A + V_D + V_{EP}$
Within pairs	$\sigma^2_{W_{MZ}}$	N_{MZ}	V_{EW}
DZ twins			
Among age groups	$\sigma^2_{A_{DZ}}$	$N_{AG}-1$	V_{ES}
Between pairs within age groups	$\sigma^2_{B_{DZ}}$	$N_{DZ}-N_{AG}$	$\frac{1}{2}V_A + \frac{1}{4}V_D + V_{EP}$
Within pairs	$\sigma^2_{W_{DZ}}$	N_{DZ}	$\frac{1}{2}V_A + \frac{3}{4}V_D + V_{EW}$

Where:
V_{ES} = environmental variance among age groups
V_{EP} = environmental variance between pairs within age groups
V_{EW} = environmental variance within pairs
V_A = additive genetic variance
V_D = dominance genetic variance
σ^{2A} = among component of variance
σ^{2B} = between component of variance
σ^{2W} = within component of variance
N_{AG} = number of age groups
N_{MZ} = number of MZ pairs
N_{DZ} = number of DZ pairs

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TABLE 16. Norwegian Twin Registry (NTR) 1915–1946. Results of Address Search and Response to Q1

Category	Number of pairs	
Total twins in NTR	22,354	
Opposite-sexed	8,169	
Same-sexed: males	6,805	14,185
Same-sexed: females	7,380	
Address search for same-sexed twins:		
Twins not found	2,024	
One or both stillborn	1,504	
One or both early death	1,753	
Previously used	404	
Total like-sexed twins found	8,500	
Response to Q1:		
Complete Yes	2,365	
Complete No	320	
Incomplete	1,671	
One stillborn or early death	638	
One late death	308	
Not a twin	117	
Returned blank	181	
No response	2,900	

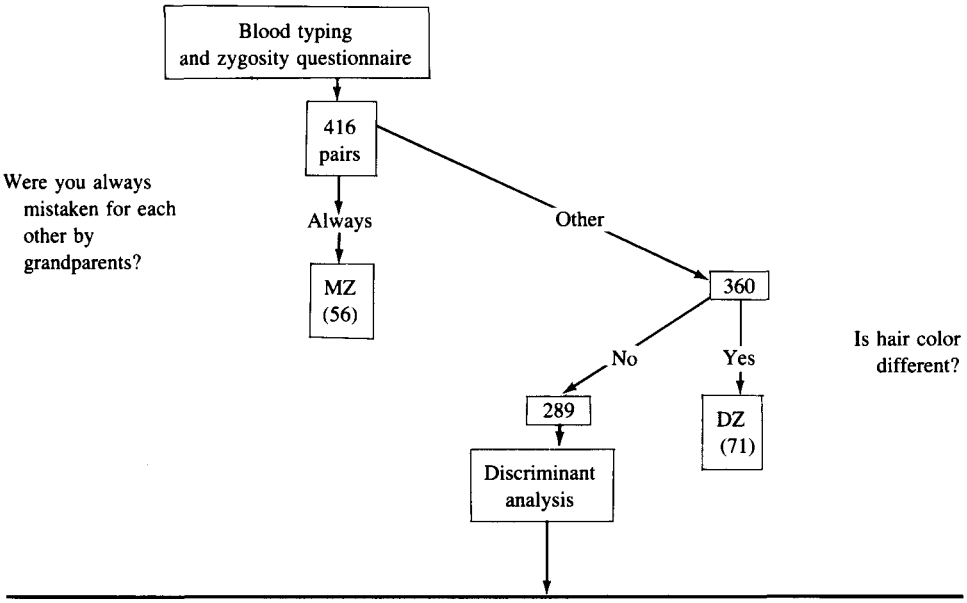
other did not, totaled 1,671. More information concerning stillbirths and early death of one of the twins was obtained from the Q1, and as a result 600 additional pairs were eliminated. In 308 pairs, one of the twins had died after the age of 20, and these were placed in a special category for a future study. In 117 cases, the respondent denied being a twin, and 181 individuals returned their questionnaires blank. In 2,900 pairs there was no response from either twin. In all, a response of some kind was received from 66% of the twin pairs contacted. It should be noted that these results change continually and that the response rate continues to increase.

The zygosity questionnaire, Q1, was found to be nearly 98% accurate in discriminating between MZ and DZ twins. Of the 416 twin pairs classified on the basis of the marker study, 199 were classified correctly as MZ by either responding that they were "always" mistaken for each other by their grandparents (56) or by a stepwise discrimination analysis (143). Based on the comparison with classification by blood markers, only three DZ pairs were misclassified as MZ. Seventy-one pairs claimed that their hair color was different and were correctly classified as DZ, and the discriminant analysis correctly classified 140 more as DZ pairs. Again, three MZ pairs were misclassified as DZ by this method. The discrimination is illustrated in Figure 3. This method was then applied to the twins responding to the questionnaires.

Q2 A and B packets were sent to 2,365 complete pairs. As of January 1980, questionnaires had been returned by both members of 573 MZ pairs and by only one member of 334 MZ pairs. Complete DZ pairs totaled 444, with 420 incomplete sets.

Twelve complete sets were discordant for zygosity and were not used. For MZ twins, 531 were usable; that is, both twins had filled out the questionnaires adequately. Usable DZ pairs totaled 384. Twin pairs were not used if one or both did not fill out the reproductive history portion but indicated that they were married, unless they specified that there were no pregnancies. These were primarily widowed or separated male twins where it appeared that the assumption of childlessness was unlikely. Those twins whose spouses were related were not included; nor were those whose answers were obviously unreliable. For all of these reasons 39 MZ and 60 DZ pairs were excluded.

The number of total pregnancies ranged from zero to nine for MZ male twins and from zero to 18 for MZ female twins. For DZ twins, the total number of pregnancies ranged from zero to eight for male twins and from zero to nine for female twins. Tables 17 through 20 show the number of total pregnancies and first-trimester spontaneous abortions for MZ and DZ twins by pair. The number of spontaneous abortions ranged from zero to seven; one spouse of a DZ male reported seven abortions in seven pregnancies. There was a total of 204 first-trimester spontaneous abortions among the 2,753 total pregnancies reported by MZ twins, giving an incidence of 7.41% (Table



Actual zygosity	N	Zygosity predicted by Q1	
		MZ	DZ
MZ	146	143	3
DZ	143	3	140

Percent of cases correctly classified: 97.92.

Fig. 3. Results of zygosity testing for Questionnaire 1.

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21). MZ male twins had an incidence of first-trimester spontaneous abortions of 7.89%, and MZ females had an incidence of 7.09%. DZ twins reported a total of 161 first-trimester spontaneous abortions among a total of 1,891 pregnancies, an incidence of 8.30% (Table 21). Male and female DZ twins had similar incidences of spontaneous abortions, 8.41% and 8.25%, respectively.

TABLE 17. Total Pregnancies Reported by MZ Twins, by Pair. MZ Female Pairs to the Right of the Diagonal (and Right of the Slash Mark). MZ Male Pairs to the Left of the Diagonal (and Left of the Slash Mark). (N Females = 312 Pairs; N Males = 219 Pairs)

Twin 1 Number of reported total pregnancies	Twin 2 Number of reported total pregnancies												
	0	1	2	3	4	5	6	7	8	9	10	11	18
0	11/14	9	11	12	10	1	0	0	0	0	0	0	0
1	3	4/7	19	10	8	3	2	1	0	0	0	0	0
2	18	17	23/30	42	31	1	3	1	1	0	0	1	0
3	8	9	30	19/21	26	12	3	1	0	0	0	0	0
4	2	2	15	8	7/7	9	6	0	1	0	0	0	1
5	1	4	7	6	4	3/1	1	2	1	0	0	0	0
6	1	1	2	2	3	0	2/1	0	1	0	0	0	0
7	0	0	0	1	2	1	0	1/0	0	0	0	0	0
8	0	0	0	0	0	1	0	0	0/0	0	0	0	0
9	0	0	1	0	0	0	0	0	0	0/0	0	0	0
10	0	0	0	0	0	0	0	0	0	0	0/0	0	0
11	0	0	0	0	0	0	0	0	0	0	0	0/0	0
18	0	0	0	0	0	0	0	0	0	0	0	0	0/0

TABLE 18. First-Trimester Spontaneous Abortions Reported by MZ Twins, by Pair. MZ Female Pairs to the Right of the Diagonal (and Right of the Slash Mark). MZ Males to the Left of the Diagonal (and Left of the Slash Mark). (N Female = 312 Pairs; N Males = 219 Pairs)

Twin 1 Number of abortions	Twin 2 Number of abortions				
	0	1	2	3	4
0	157/233	49	17	0	0
1	46	1/6	6	1	0
2	9	2	0/0	0	0
3	2	1	0	0/0	0
4	0	1	0	0	0/0

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TABLE 19. Total Pregnancies Reported by DZ Twins, by Pair. DZ Female Pairs to the Right of the Diagonal (and Right of the Slash Mark). DZ Male Pairs to the Left of the Diagonal (and Left of the Slash Mark). (N Female = 241 Pairs; N Males = 143 Pairs)

Twin 1 Number of reported total pregnancies	Twin 2 Number of reported total pregnancies									
	0	1	2	3	4	5	6	7	8	9
0	10/4	4	20	11	4	5	1	0	3	0
1	1	1/2	19	15	6	2	1	0	0	0
2	17	4	19/29	28	11	12	1	0	0	0
3	4	3	21	10/14	19	6	2	0	0	1
4	6	5	11	11	2/7	3	3	1	1	0
5	2	2	2	2	3	0/5	0	1	0	0
6	0	1	0	2	1	0	0/0	0	0	0
7	0	0	1	0	0	1	0	0/0	0	0
8	0	1	0	0	0	0	0	0	0/0	0
9	0	0	0	0	0	0	0	0	0	0/0

TABLE 20. First-Trimester Spontaneous Abortions Reported by DZ Twins, by Pair. DZ Female Twins to the Right of the Diagonal (and Right of the Slash Mark). DZ Male Pairs to the Left of the Diagonal (and Left of the Slash Mark). (N Females = 241 Pairs; N Males = 143 Pairs)

Twin 1 Number of abortions	Twin 2 Number of abortions					
	0	1	2	3	4	7
0	102/174	40	11	1	0	0
1	33	2/8	5	1	1	0
2	3	1	0/0	0	0	0
3	1	0	0	0/0	0	0
4	0	0	0	0	0/0	0
7	1	0	0	0	0	0/0

TABLE 21. Number of First-Trimester Spontaneous Abortions Reported by MZ and DZ Twins (N = 1,830 Individuals)

	MZ twins			DZ twins		
	Males	Females	Total	Males	Females	Total
Total pregnancies	1,103	1,650	2,753	666	1,225	1,891
Spontaneous abortions	87	117	204	56	105	161
Incidence	0.0789[a]	0.0709[a]	0.0741[c]	0.0841[b]	0.0857[b]	0.0851[c]

[a] $\chi^2 = 0.629$, df = 1; $P > 0.05$.
[b] $\chi^2 = 2.29$, df = 1; $P > 0.05$.
[c] $\chi^2 = 3.11$, df = 1; $P > 0.05$.

Table 22 summarizes the outcome of all pregnancies for MZ twins. There were a total of 970 live-born deliveries, including twins, for MZ males, and 1,445 for MZ females. A total of 28 pregnancies ended in a late spontaneous abortion, and 73 ended by an induced abortion. Twenty-four pregnancies ended in a stillbirth and nine pregnancies were reported to be ectopic. Eighteen twin births were reported; seven same-sexed male pairs, five same-sexed female pairs, six opposite-sexed pairs.

The outcome of all pregnancies for DZ twins is given in Table 23. There was a total of 586 live-borns for DZ females. A total of 21 pregnancies ended in a late spontaneous abortion, and 56 ended by induced abortion. Twenty pregnancies resulted in a stillbirth, and four pregnancies were reported to be ectopic. Twenty-five twin births were reported; five same-sexed female pairs, four same-sexed male pairs, and 16 opposite-sexed pairs.

When early and late spontaneous abortions were combined, a total of 97 abortions were reported by MZ males and 135 abortions by MZ females (Table 24). This increased the incidence of abortion to 8.79% for MZ males and 8.18% for MZ females.

TABLE 22. Distribution of the Outcome of Pregnancy Reported by MZ Twin Individuals

Outcome of pregnancy	MZ males (N = 382)	MZ females (N = 553)	Total MZ
Live-born: Males	516	718	1,234
Females	449	714	1,163
First-trimester spontaneous abortions	87	117	203
Late spontaneous abortions	10	18	28
Induced abortions	26	47	73
Stillbirths	5	19	24
Twin births: Same-sexed females	2	5	7
Same-sexed males	2	3	5
Opposite-sexed	1	5	6
Ectopic pregnancies	5	4	9
Total	1,103	1,650	2,753

TABLE 23. Distribution of the Outcome of Pregnancy Reported by DZ Twin Individuals

Outcome of pregnancy	DZ males (N = 236)	DZ females (N = 426)	Total DZ
Live-born: Males	298	532	830
Females	283	491	774
First-trimester spontaneous abortions	56	105	161
Late spontaneous abortions	4	17	21
Induced abortions	15	41	56
Stillbirths	5	15	0
Twin births: Same-sexed females	1	4	5
Same-sexed males	1	3	4
Opposite-sexed	3	13	16
Ectopic pregnancies	0	4	4
Total	666	1,225	1,891

TABLE 24. Incidence of First-Trimester and Late Spontaneous Abortions Combined (N = 1,830 Individuals)

	MZ twins			DZ twins		
	Males	Females	Total	Males	Females	Total
First-trimester spontaneous abortions	87	117	204	56	105	161
Late spontaneous abortions	10	18	28	4	17	2
Total spontaneous abortions	97	135	232	60	122	182
Total pregnancies	1,103	1,650	2,753	666	1,225	1,891
Incidence of total spontaneous abortions	0.0879[a]	0.0818[a]	0.0843[c]	0.0900[b]	0.0995[b]	0.0962[c]

[a]$\chi^2 = 0.152$, df = 1; $P > 0.05$.
[b]$\chi^2 = 0.352$, df = 1; $P > 0.05$.
[c]$\chi^2 = 1.95$, df = 1; $P > 0.05$.

TABLE 25. Incidence of Twin Births Reported by MZ and DZ Twins (N = 1,830 Individuals)

	MZ twins		DZ twins	
Births	Males	Females	Males	Females
Twin births	5	13	5	20
Singleton births	965	1,432	581	1,023
Total births	970	1,445	586	1,043
Incidence of twin births	0.00515	0.00899	0.00853	0.0192

TABLE 26. Twin Births Reported by MZ and DZ Twins: Analysis of χ^2

Source of variation	df	χ^2
Zygosity	1	5.63*
Sex	1	4.25*
Sex by zygosity	1	0.73
Overall	3	10.61*

*Significant at P = 0.05.

For DZ twins, early and late spontaneous abortions totaled 60 (9.0%) and 122 (9.95%) for male and female twins, respectively (Table 24).

Table 25 gives the incidence of twin births among live births for the various groups. MZ males had a twinning rate of approximately 0.005, and MZ females had a rate of approximately 0.009. DZ males were found to have an incidence of approximately 0.008 twin births, and DZ females were somewhat higher, 0.019. Contingency χ^2s of 5.63 and 4.25 indicated that DZ twins and females of both zygosities, respectively, had significantly more twin births, with no evidence for a sex effect within zygosities.

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A threefold classification of twin pairs by marital status (never married, married without pregnancies, and married with pregnancies) is given in Table 27 for male and female MZ twins and in Table 28 for male and female DZ twins. A contingency χ^2 analysis showed that the members of MZ male pairs and MZ female pairs were not randomly distributed with respect to marital status, the discrepancy being largely attributable to an excess of pairs concordant for marital status (χ^2 = 45.87 and 8.77, 4 df, respectively) (Table 30). The distribution of DZ male twin pairs also differed from expectation (χ^2 = 13.85, 4 df), but DZ female pairs did not show a significant departure from a random association of marital status for members of a pair (χ^2 = 3.85, 4 df). A heterogeneity χ^2 was performed and the results are shown in Table 30. Zygosity was found to account for a significant portion of the heterogeneity (χ^2 = 33.14, 1 df) among the four sex-zygosity groups. While sex alone did not have a significant overall effect (χ^2 = 2.69, 1 df), the interaction of sex and zygosity was highly significant (χ^2 = 12.32, 1 df), resulting from the fact that, among MZ twins, females showed a greater departure from random association of marital status than MZ males, whereas the reverse was true for DZ twins.

TABLE 27. Marital Status of MZ Twins by Sex

	Twin 1							
	Never married		Married without pregnancies		Married with pregnancies		Total	
Twin 2	Males	Females	Males	Females	Males	Females	Males	Females
Never married	6	12	0	0	6	17	12	29
Married without pregnancies	1	1	4	2	11	12	16	15
Married with pregnancies	11	6	6	8	174	254	191	268
Total	18	19	10	10	191	283	219	312

TABLE 28. Marital Status of DZ Twins by Sex

	Twin 1							
	Never Married		Married without pregnancies		Married with pregnancies		Total	
Twin 2	Males	Females	Males	Females	Males	Females	Males	Females
Never married	5	4	1	0	14	27	20	31
Married without pregnancies	3	0	1	0	4	4	8	4
Married with pregnancies	8	11	4	6	103	189	115	206
Total	16	15	6	6	121	220	143	241

TABLE 29. MZ and DZ Twins Concordant and Discordant for Marital Status

Zygosity	Concordant	Discordant	Total
MZ	489	42	531
DZ	320	64	384
Total	809	106	915

χ^2 = 16.83, df = 1; $P < 0.001$.

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TABLE 30. Analysis of the Effects of Sex and Zygosity on Marital Status

Groups (df = 4)	χ^2	Heterogeneity χ^2		
1. MZ males	45.87*	Total χ^2 = 145.34 (1+2+4+5)		
2. MZ females	81.77*			
3. MZ pooled male and female	115.95*	Pooled χ^2 = 97.19 Heterogeneity χ^2 = 48.15*		
4. DZ males	13.85*	Source	d	χ^2
5. DZ females	3.85	Zygosity	1	33.14* (3+6)−9
		Sex	1	2.69 (7+8)−9
6. DZ pooled male and female	14.38*	Sex and zygosity	1	12.32*
		Heterogeneity	3	48.15
7. MZ and DZ males	45.83*			
8. MZ and DZ females	54.05*			
9. Pooled total	97.19*			

*Significant at p = 0.05.

4.2. Analysis of First-Trimester Spontaneous Abortions by the Qualitative Half-Sib Model

Tables 31–34 give the maximum likelihood estimates of the five risk parameters for first trimester spontaneous abortion. A total of 174 MZ male and 254 MZ female half-sib families were used in the initial analysis (Model 1A). The recurrence risk for spontaneous abortion if the mother was at high risk, a, was estimated to be over 13%, whereas b, the recurrence risk if the father is high risk, was so low that it converged to a bound set by the MAXLIK program. The sporadic risk, w, also converged to zero. The estimate of the frequency of high risk individuals in the population, p, was over 54%. A second analysis was conducted after removing one half-sib family with an extreme of 18 pregnancies (Model 1B). This analysis resulted in similar estimates of the parameters, as shown in Table 31. The data were then reanalyzed eliminating five families with three or more spontaneous abortions (Model 1C). The parameter estimates changed slightly in this analysis with a, the estimate of maternal risk falling to approximately 12%. The paternal risk, b, remained at zero, and the population frequency rose to approximately 57%.

A comparison of the likelihoods obtained under Model 1A, where c was constrained to be a function of a and b, and Model 2 in which all parameters were fixed at zero except for the sporadic risk, w, is given in Table 32. With the "genetic" and "maternal" risk eliminated, Model 2 estimated the sporadic risk to be approximately 7.6%. The likelihood ratios of these two models had a χ^2 of 25.2 (3 df), indicating that Model 1A had a significantly better fit than Model 2. Table 32 also gives the estimates obtained under Model 3, where parameter a was set to equal b. The χ^2 test for goodness of fit was 5.0 (1 df), indicating that Model 1A was more likely than Model 3.

Table 33 gives the estimates obtained when all parameters were independent (Model 4) as compared with Model 1A in which c was dependent on a and b. There was no significant difference in the goodness of fit of these two models.

TABLE 31. Analysis of First-Trimester Abortions Reported by MZ Twins: Results of Maximum Likelihood Estimation With $c = a + b$

	Model 1A: All females included		Model 1B: One family omitted*		Model 1C: Five families omitted**	
Parameter	Estimate	SE	Estimate	SE	Estimate	SE
a	0.13467	0.01528	0.13382	0.01556	0.11939	0.01540
b	0.00000	—	0.00000	—	0.00000	—
c	0.13467	0.01528	0.13382	0.01556	0.11939	0.01540
w	0.00000	—	0.00000	—	0.00000	—
p	0.54271	0.05980	0.54230	0.06080	0.57135	0.07024
Log likelihood	−671.30		−660.0		−622.69	

*Half sibship containing one woman with 18 pregnancies omitted.

**Five half-sibships containing women with three or more spontaneous abortions omitted.

TABLE 32. Analysis of First-Trimester Abortions Reported by MZ Twins: Results of a Comparison of Likelihoods of Maximum Likelihood Estimations

	Model 1A: All families (c = a + b)		Model 2: Restricted model with a = b = c = p = 0		Model 3: Restricted model with a = b and c = a + b	
Parameter	Estimate	SE	Estimate	SE	Estimate	SE
a	0.13467	0.01528	0.00000	—	0.10387	0.01764
b	0.00000	—	0.00000	—	0.10387	0.01764
c	0.13467	0.01528	0.00000	—	0.19696	0.03161
w	0.00000	—	0.07557	0.00523	0.00000	—
p	0.54271	0.05980	0.00000		0.35518	0.06117
Log likelihood	−671.30		−683.90		−673.81	

Calculation of likelihood ratio $\chi^2 = -2 \log \frac{\text{likelihood 1}}{\text{likelihood 2}}$.

A. Model 1 vs Model 2
$-673.90 - (-671.30) = -12.6$
$\chi^2 = (-12.6) \times (-2) = 25.2$, df = 3, significant at $P < 0.05$.

B. Model 1 vs Model 3
$-673.81 - (-671.30) = -2.50$
$\chi^2 = (-2.50) \times (-2) = 5.0$, df = 1, significant at $P < 0.05$.

Table 34 gives the estimates obtained when the analysis was done separately on twins less than or equal to 44 years of age (Model 1D) and those over the age of 44 years (Model 1E). By adding the likelihoods obtained in these models and subtracting them from those obtained for the whole population (Model 1A), an indication of heterogeneity is calculated. A χ^2 of 0.30 (1 df) was found indicating that the two subpopulations did not have a significant amount of heterogeneity.

4.3. Other Reproductive Variables

4.3.1. Age of menarche. There was a small but significant difference in the mean age of menarche between MZ female twins (13.75 years) and the spouses of male MZ twins (13.36 years), as shown in Table 35. The same pattern was found in the DZ group, where the female DZ twins had a mean age of menarche of 13.88 years and the spouses of male DZ twins had a mean of 13.48 years. There was no significant difference between MZ and DZ female twins or between the spouses of male MZ or DZ male twins for the mean age of menarche (Table 35).

A comparison of the mean within-pair difference in age at menarche between female twins and the spouses of male twins is also shown in Table 35. A mean within-pair difference of 0.62 years was found for MZ female twins, which was significantly smaller than that found between the spouses of MZ male twins (1.39 years). There was also a significant difference between DZ female twins and the spouses of DZ male twins for the mean within-pair difference in age of menarche (1.18 years and 1.54 years, respectively). No significant difference was found between the spouses of male MZ or DZ twins for the mean difference in age of menarche (1.39 years and 1.54

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TABLE 33. Analysis of First-Trimester Abortions Reported by MZ Twins: Results of a Comparison of Likelihoods for Models With and Without an Independent c Parameter

	Model 4: All families, all independent parameters		Model 1A: All families, c = a + b		Calculation of $-2\frac{\text{likelihood 1}}{\text{likelihood 2}}$
Parameter	Estimate	SE	Estimate	SE	
a	0.13468	0.05248	0.13467	0.01528	$(-671.31)-(671.30)=-0.01$
b	0.00000	—	0.00000	—	$\chi^2=(-0.01)\times(-2)=0.02$
c	0.13467	0.04546	0.13467	0.01528	df = 1, $P > 0.05$
w	0.54270	0.05979	0.54271	−0.5980	
Log likelihood	−671.31		−671.30		

TABLE 34. Analysis of First-Trimester Abortions Reported by MZ Twins: Results of Analyzing Younger and Older Twins Separately (c = a + b)

	Model 1D: Younger twins ≤ 44 years		Model 1E: Older twins > 44 years		Model 1A: All families	
Parameter	Estimate	SE	Estimate	SE	Estimate	SE
a	0.13619	0.02217	0.13280	0.02107	0.13467	0.01528
b	0.00000	—	0.00000	—	0.00000	—
c	0.13619	0.02217	0.13280	0.02107	0.13467	0.01528
w	0.00000	—	0.00000	—	0.00000	—
p	0.56009	0.08894	0.52755	0.08109	0.54271	0.05980
Log likelihood	−333.40		−337.60		−671.30	

Heterogeneity χ^2: $(-333.40) + (-337.60) = -671.00$.
$\chi^2 = (-671.30) - (-671.00) = 0.30$.
df = 1; $P > 0.05$.

TABLE 35. Age of Menarche. Comparison Between Female Twins and the Spouses of Male Twins and Between MZ and DZ Twins for the Mean Age of Menarche and the Mean Within-Pair Difference in Age of Menarche*

Zygosity	Spouses of male twins N	Spouses of male twins Mean	Female twins N	Female twins Mean	Probability of t for female twins vs spouses of male twins
MZ twins					
Mean age	296	13.36 ± 0.08	595	13.75 ± 0.56	0.0001
Mean difference	115	1.39 ± 0.10	290	0.62 ± 0.15	0.0001
DZ twins					
Mean age	196	13.48 ± 0.10	458	13.88 ± 0.06	0.0004
Mean difference	75	1.54 ± 0.13	217	1.18 ± 0.08	0.0166
Probability of t for MZ and DZ twins or spouses					
Mean age		0.3113		0.1322	
Mean difference		0.3574		0.0001	

*Calculated as absolute difference.

TABLE 36. Age of Menarche. Spearman Correlations for the Age of Menarche in MZ and DZ Female Twins and the Spouses of Male Twins

Zygosity	Spouses of male twins	Female twins
MZ twins		
r	0.00066	0.70521
p	0.9440	0.0001
N	115	290
DZ twins		
r	−0.08724	−0.28743
p	0.4567	0.0001
N	75	217

years, respectively), but a significant difference between MZ and DZ female twins was found (Table 35). MZ female twins had a significantly smaller mean within-pair difference than DZ female twins.

Spearman correlations of age of menarche between female twins and between the unrelated spouses of male twins are shown in Table 36 for both MZ and DZ twins. No significant correlation was seen between the spouses of male twins for either MZ or DZ twins. MZ twins had a correlation coefficient of 0.70, whereas DZ females twins had a lower but significant correlation coefficient of 0.029.

The results of an analysis of variance for the age of menarche in female twins of all ages are shown in Table 37. The variation within MZ pairs was significantly less than in DZ pairs ($F = 2.63$, $P < 0.01$). The female twins were divided by age (≤ 44 years and > 44 years), and analyses of variance were perfomred on each group separately (Table 38). While in both age groups MZ pairs had significantly less within-pair variation, the within-pair mean square for DZ female twins in the younger age group increased considerably. When various genetic and environmental models were fit to the data pooled over all age groups (Table 39), a simple additive genetic and random environmental model fit the data. Separate analyses on younger and older twins yielded

TABLE 37. Analysis of Variance for the Age of Menarche in MZ and DZ Female Twins

	MZ		DZ	
Source	df	Mean square	df	Mean square
Among pairs	289	2.8899	216	2.5248
Within pairs	290	0.4983	217	1.3111

$F_{(within)} = 2.6311$, significant at $P < 0.01$.

TABLE 38. Analysis of Variance for the Age of Menarche in MZ and DZ Female Twins Divided by Age Group (≤ 44 Years; > 44 Years)

Age		MZ		DZ	
group	Source	df	Mean square	df	Mean square
<44	Among pairs	155	2.7289	112	1.9068
	Within pairs	156	0.4039	113	1.4646
>44	Among pairs	133	2.8231	103	2.8745
	Within pairs	134	0.6082	104	1.1442

$F_{(within,\ \leq 44)} = 3.626$, significant at $P < 0.01$.
$F_{(within,\ > 44)} = 1.880$, significant at $P < 0.01$.

TABLE 39. Analysis of the Age of Menarche by Weighted Least-Squares Procedure Using MZ and DZ Female Twins; Analysis Done on Pooled Data and on Older and Younger Twins Separately

		Divided by older and younger	
Component	All ages pooled	≤ 44 years	> 44 years
V_A	1.3166 ± 0.0714	1.3175 ± 0.0714	1.2670 ± 0.1041
V_D			
V_{EW}	0.5157 ± 0.0302	0.4479 ± 0.0319	0.6014 ± 0.0497
V_{EP}			
χ^2	2.64	2.64	2.09
df	2	2	2
P	0.2667	0.0059	0.3515

different results; the simple $V_A - V_{EW}$ model was rejected in the younger twin data, but fit the older twin data. An additional environmental component, V_{ES}, was added to account for variation between age groups. The results of nested analyses of variance for the age of menarche in female MZ and DZ twins stratified into two age groups (those born 1915–1935 and 1936–1946) are shown in Table 40. A model with only a random environmental component was rejected ($\chi^2 = 253.7$, 5 df), as was a simple additive genetic and random environmental model ($\chi^2 = 101.72$, 4 df) (Table 42). A model that included the environmental component for age stratification, V_{ES}, fit the data, but the standard errors of the estimates were large. The data were again divided into seven age groups: birth years 1915–1919, 1920–1924, 1925–1929, 1930–1934,

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TABLE 40. Nested Analysis of Variance of the Age of Menarche in MA and DZ Female Twins, Stratified Into Two Age Groups: Birth Years 1915–1935 and 1936–1946

Source	df	MZ mean square	df	DZ mean square
Among age groups	1	36.73433	1	35.71154
Between pairs within age groups	288	2.74241	215	2.37030
Within pairs	290	0.49828	217	1.31106

TABLE 41. Nested Analysis of Variance of the Age of Menarche in MZ and DZ Female Twins, Stratified Into Seven Age Groups

Source	df	MZ mean square	df	DZ mean square
Among age groups	6	7.06060	6	8.63683
Between pairs within age groups	283	2.80151	210	2.35016
Within pairs	290	0.49828	217	1.31106

TABLE 42. Analysis of the Age of Menarche by Weighted Least-Squares Procedure Using MZ and DZ Female Twins, With Stratification Into Two Age Groups

	Estimates ± SE			
Component	Model 1	Model 2	Model 3	Model 4
V_A		2.0986 ± 0.1157	1.2493 ± 0.0697	0.6860 ± 0.2955
V_D				0.5572 ± 0.2928
V_{EW}	0.3587 ± 0.1597	0.4895 ± 0.0279	0.5210 ± 0.0365	0.5040 ± 0.0297
V_{EP}				
V_{ES}			0.1376 ± 0.1057	0.1382 ± 0.1059
χ^2	253.71	101.72	3.52	1.37
df	5	4	3	2
P	0.0000	0.0000	0.3179	0.5048

TABLE 43. Analysis of the Age of Menarche by Weighted Least-Squares Procedure Using MZ and DZ Female Twins, With Stratification Into Seven Age Groups

	Estimates ± SE			
Component	Model 1	Model 2	Model 3	Model 4
V_A		1.4538 ± 0.0784	1.2526 ± 0.0703	0.6674 ± 0.2988
V_D				0.5766 ± 0.2956
V_{EW}	2.5270 ± 0.0944	0.5077 ± 0.0295	0.5206 ± 0.0306	0.5034 ± 0.0296
V_{EP}				
V_{ES}			0.0825 ± 0.0306	0.0853 ± 0.0364
χ^2	147.95	24.80	3.62	1.37
df	5	4	3	2
P	0.000	0.000056	0.3055	0.5048

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1935–1939, 1040–1944, and 1945–1946. The results of a nested analysis of variance for age of menarche with stratification into seven age groups are shown in Table 41. The various models fit to the data are shown in Table 43. Models with only a random environment component or additive genetic and random environmental components were rejected (χ^2 = 147.95, 5 df, and χ^2 = 24.80, 4 df, respectively). Models with the environmental component for age stratification fit the data, and the estimates of V_{ES} had smaller standard errors than those found in the two age group models.

4.3.2. Age of menopause. The average age of menopause is given in Table 44 for MZ and DZ female twins and for the spouses of male twins. For this variable, there was no significant difference between the groups. The mean difference between MZ female twins for the age of menopause was 2.47 years, which was significantly less than the 5.63 years found for the spouses of MZ male twins (Table 44). On the other hand, no significant difference was found for the mean difference between DZ female twins (3.64 years) and the spouses of male DZ twins (2.78 years). The mean difference for MZ female twins was found to be significantly less than that for DZ female twins (P = 0.0267) (Table 44). The spouses of DZ male twins had a significantly smaller mean difference when compared with the spouses of MZ male twins (P = 0.0475), although the sample size was quite small.

The correlation between MZ females for age of menopause was 0.62 whereas for DZ females it was approximately 0.37 (Table 45). Both correlations were significantly greater than zero, in contrast to the negative correlations found for the spouses of MZ and DZ male twins.

The model-fitting procedure previously described was also used to examine the nature of factors important in the determination of age of menopause. Table 46 gives the among and within the mean squares for the MZ and DZ twin pairs. An F-test of the within-pair variances was not significant. However, the model-fitting procedure indicated that a model was additive genetic, V_A, and the within environmental component, V_{EW}, fit the data quite well (Table 47). The sample sizes were too small to divide by age.

TABLE 44. Age of Menopause. Comparison Between Female Twins and the Spouses of Male Twins, and Between MZ and DZ Twins for Mean Age of Menopause and Mean Within-Pair Difference in Age of Menopause*

	Spouses of male twins		Female twins		Probability of t for female twins vs spouses of male twins
Zygosity	N	Mean	N	Mean	
MZ twins					
Mean age	55	47.36 ± 0.74	229	47.89 ± 0.33	0.4927
Mean difference	16	5.63 ± 1.16	104	2.47 ± 0.34	0.0019
DZ twins					
Mean age	41	48.73 ± 0.73	179	47.35 ± 0.36	0.0966
Mean difference	9	2.78 ± 0.70	75	3.64 ± 0.37	0.4533
Probability of t for MZ and DZ twins or spouses					
Mean age		0.2028		0.2738	
Mean difference		0.0475		0.0267	

*Calculated as absolute difference.

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TABLE 45. Age of Menopause. Spearman Correlations for the Age of Menopause in MZ and DZ Female Twins and the Spouses of Male Twins

Zygosity	Spouses of male twins	Female twins
MZ twins		
r	−0.58912	0.62466
P	0.0163	0.0001
N	16	104
DZ twins		
r	−0.67489	0.36605
P	0.0461	0.0012
N	9	75

TABLE 46. Analysis of Variance for Age of Menopause Using MZ and DZ Female Twins

	MZ		DZ	
Source	df	Mean square	df	Mean square
Among pairs	103	28.2273	74	22.3303
Within pairs	104	9.2163	75	12.1400

$F_{(within)} = 1.317$, $P > 0.05$.

TABLE 47. Analysis of the Age of Menopause by Weighted Least-Squares Procedure. All Ages Included

Component	Estimate ± SE
V_A	9.116 ± 1.136
V_D	
V_{EW}	8.930 ± 0.802
V_{EP}	
χ^2	5.13
df	2
P	0.7739

TABLE 48. Age at Marriage. Comparison of MZ and DZ Twins and Their Spouses for Mean Age at Marriage

	MZ twins			DZ twins			Probability of t for MZ vs DZ twins and spouses
Sex	N	Mean ± SE	SD	N	Mean ± SE	SD	
Male twins							
Twins	388	26.39 ± 0.23	4.48	240	26.91 ± 0.32	5.01	0.1505
Spouses	365	23.04 ± 0.20	3.81	224	23.28 ± 0.26	3.81	0.4580
Female twins							
Twins	535	23.70 ± 0.17	3.89	407	24.13 ± 0.20	4.04	0.1014
Spouses	404	26.21 ± 0.27	4.76	307	27.18 ± 0.29	5.11	0.0092

4.3.3. Age at marriage. The mean age at marriage for MZ male twins was 26.39 years and 26.21 years for the male spouses of MZ female twins. DZ males were not significantly different from MZ male twins for age at marriage, 26.91 years, but the male spouses of DZ female twins were significantly older than their MZ counterparts (27.18 years and 26.21 years, respectively) (Table 48). The mean age at marriage for MZ female twins was 23.70 years, which was not significantly different from the mean for DZ female twins, 24.13 years (Table 48). The female spouses of male twins had a mean age of marriage of 23.04 years for MZ twins and 23.48 years for DZ twins and were not significantly different.

There was no significant difference between male and female MZ twins for the mean within-pair difference in age at marriage (Table 49); the same was found for DZ male and female twins, although for both MZ and DZ twins the females were characterized by smaller differences. The mean difference for the spouses of female DZ twins (4.92 years) was significantly higher than that for the spouses of male DZ twins (3.09 years). No significant difference was found between MZ and DZ twins when the mean within-pair difference between cotwins was compared, as shown in Table 49.

The Spearman correlation in age of marriage between twins was significantly greater than zero for both MZ and DZ groups, and it was also significant for the spouses of both MZ and DZ twins (Table 50). The marital correlation and the twin-spouse correlation (twin 1 and spouse 2 and vice versa) were both significant for all groups.

4.3.4. Parental age at first birth. The mean age at the birth of the first child for the various groups is given in Table 51. There was no significant difference between any of the groups for this variable. The mean within-pair difference between cotwins was 3.29 years for MZ female twins, which was significantly lower than the difference between MZ male twins, 4.49 years (Table 52). However, the mean within-pair difference between spouses of male and female MZ twins was not significantly different.

TABLE 49. Age at Marriage. Comparison of Male and Female Twins and Their Spouses, and MZ and DZ Twins and Their Spouses for the Mean Difference in Age at Marriage*

Zygosity	Male twins N	Male twins Mean	Female twins N	Female twins Mean	Probability of t for male vs female twins, spouses, and husband-wife
MZ twins—Mean difference					
Twins	180	3.43 ± 0.13	244	2.93 ± 0.21	0.1327
Spouses	162	3.27 ± 0.23	158	3.99 ± 0.31	0.0671
Husband-wife	370	3.61 ± 0.15	386	3.60 ± 0.16	0.9841
DZ twins—Mean difference					
Twins	104	3.58 ± 0.38	175	3.52 ± 0.26	0.8972
Spouses	95	3.09 ± 0.33	108	4.92 ± 0.42	0.0007
Husband-wife	216	3.48 ± 0.20	288	3.91 ± 0.22	0.1229
Probability of t for MZ and DZ twins, spouses, and husband-wife					
Twins		0.7375		0.0740	
Spouses		0.6512		0.0715	
Husband-wife		0.5428		0.2527	

*Calculated as the absolute difference.

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TABLE 50. Age at Marriage. Spearman Correlations for Twins and Spouses for the Age at Marriage

	MZ		DZ	
Correlation	Males	Females	Males	Females
Twin				
r	0.40545	0.41559	0.39620	0.32349
P	0.0001	0.0001	0.0001	0.0001
N	180	244	104	175
Marital				
r	0.61344	0.56755	0.55155	0.56357
P	0.0001	0.0001	0.0001	0.0001
N	365	407	224	307
Spouse				
r	0.34219	0.30661	0.31587	0.21007
P	0.0001	0.0001	0.0018	0.0291
N	162	158	95	108
Twin-spouse				
r	0.23421	0.19999	0.25939	0.19848
P	0.0001	0.0001	0.0002	0.0001
N	340	376	198	264

TABLE 51. Parental Age at First Birth. Comparison of MZ vs DZ Twins for the Mean Age at First Birth

	MZ twins			DZ twins			
Sex	N	Mean ± SE	SD	N	Mean ± SE	SD	P
Male twins							
Twins	314	27.07 ± 0.28	4.98	223	27.60 ± 0.31	4.65	0.1823
Spouses	309	24.11 ± 0.26	4.62	219	24.45 ± 0.28	4.25	0.3414
Female twins							
Twins	481	24.60 ± 0.19	4.21	390	24.91 ± 0.23	4.55	0.2846
Spouses	359	27.40 ± 0.26	4.96	290	28.08 ± 0.33	5.53	0.0884

TABLE 52. Parental Age at First Birth. Comparison of Male and Female Twins and Their Spouses and MZ and DZ Twins and Their Spouses for the Mean Within-Pair Difference in Age at First Birth*

	Male twins		Female twins		Probability of t for male vs female twins and spouses
Zygosity	N	Mean	N	Mean	
MZ twins—Mean difference					
Twins	127	4.49 ± 0.32	199	3.29 ± 0.22	0.0032
Spouses	123	3.99 ± 0.31	125	4.58 ± 0.33	0.1960
DZ twins—Mean difference					
Twins	91	3.67 ± 0.32	159	3.72 ± 0.25	0.8983
Spouses	89	3.87 ± 0.36	99	5.18 ± 0.39	0.0144
Probability of t for MZ vs DZ twins and spouses					
Twins		0.0922		0.2010	
Spouses		0.7909		0.2422	

*Calculated as the absolute difference.

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Table 52 also shows the results for DZ twins. There was no significant difference between the male and female DZ twins (3.67 years and 3.72 years, respectively), but the mean difference in age for the female spouses of male DZ twins (3.87) years) was significantly smaller than that for the male spouses of female DZ twins (5.18 years). There was no significant difference between MZ and DZ twins for either male or female twins, for the mean age at first birth, or the mean within-pair difference in age at first birth (Table 52).

The Spearman correlation between twins and the spouses for the age at first birth are shown in Table 53. The only group not significantly correlated was the male spouses of female DZ twins. Female MZ twins had a correlation coefficient of 0.25. For DZ twins, the male twins actually had a higher correlation coefficient, 0.41, than the female DZ twins, 0.29.

4.3.5. Interval between marriage and first birth. The mean interval between marriage and the birth of the first child was 1.48 years for MZ female twins and 1.32 years for MZ male twins; these were not significantly different. The mean interval for DZ male and female twins also were not significantly different. These values are given in Table 54.

No significant difference in male and female twins are found for mean within-pair difference in the interval for either MZ or DZ twins (Table 54). There were no significant differences between MZ and DZ twins for either mean interval or mean within-pair difference in interval (Table 54). Cotwin, marital, and spousal correlations for the interval between marriage and first birth are shown in Table 55. Both female MZ twins ($r = 0.16076$) and DZ male twins ($r = 0.255$) had Spearman correlation coefficients significantly greater than zero.

4.3.6. Age as a factor. A significant decrease in the age of menarche was seen in the "younger" ($\leq$ 44 years of age) age group of female twins and the female spouses of male twins for both MZ and DZ groups compared with the "older" group ($>$ 44 years of age) (Table 56). The characteristic of the female twins, both MZ and DZ, to have a later age of menarche was seen in both the younger and older age groups.

TABLE 53. Parental Age at First Birth. Spearman Correlations for Twins and Spouses

	MZ		DZ	
Correlations	Males	Females	Males	Females
Twins				
r	0.25115	0.41979	0.41243	0.28961
P	0.0044	0.0001	0.0001	0.0002
N	127	199	91	159
Spouses				
r	0.25491	0.24907	0.30217	0.15407
P	0.0044	0.0049	0.0040	0.1279
N	123	126	89	99
Marital				
r	0.70600	0.65425	0.63843	0.61103
P	0.0001	0.0001	0.0001	0.0001
N	309	359	219	209

TABLE 54. Interval Between Marriage and First Birth. Comparison of Males and Females, MZ and DZ, for the Mean Interval and Mean Within-Pair Difference in Interval Between Marriage and First Birth*

Zygosity	Male twins		Female twins		Probability of t for male twins vs female twins
	N	Mean	N	Mean	
MZ twins					
Mean interval	299	1.32 ± 0.10	33	1.48 ± 0.13	0.3316
Mean difference	114	1.54 ± 0.21	166	1.19 ± 0.11	0.1491
DZ twins					
Mean interval	209	1.49 ± 0.11	339	1.15 ± 0.10	0.3750
Mean difference	80	1.49 ± 0.10	125	1.31 ± 0.13	0.4650
Probability of t for MZ vs DZ twins					
Mean interval		0.2274		0.8448	
Mean difference		0.8706		0.4650	

*Calculated as the absolute difference.

TABLE 55. Interval Between Marriage and First Birth. Spearman Correlations for Male and Female Twins and Spouses

Zygosity	Male twins	Female twins
MZ twins		
r	0.09129	0.16076
P	0.3341	0.0385
N	114	166
DZ twins		
r	0.25566	−0.00225
P	0.0221	0.9801
N	80	125

The age of marriage, given in Table 57, also decreased in younger twins by an average of about 2-1/3 years in all categories. Similarly, the age at the birth of the first child was significantly lower in the younger groups (Table 58); however, there was no significant difference between age groups for the interval between marriage and the birth of the first child (Table 59).

A significant difference was found between the age groups for the number of total pregnancies reported (Table 60). The younger age group of MZ males had a mean of 2.26 pregnancies, and the older group had a mean of 2.82 pregnancies. Younger MZ females reported a mean of 2.51 pregnancies, and older MZ females had a mean of 2.79 ($P = 0.0408$). Although the means were smaller in the younger age DZ group, there was no significant difference in the total number of pregnancies for either male or female DZ twins. The same pattern was seen when only "unaffected" pregnancies were examined. Younger MZ males and females had a smaller mean number of unaffected pregnancies, although it was significant only in the MZ males ($P = 0.0001$ for males and 0.0595 for females). Again, the DZ twins showed no significant difference

TABLE 56. Age of Menarche. Comparison of Younger (≤ 44 Years) and Older (> 44 Years) Female Twins and the Spouses of Male Twins for Mean Age of Menarche

Zygosity	Younger twins N	Younger twins Mean	Older twins N	Older twins Mean	Probability of t for older vs younger twins and spouses
MZ twins					
Spouses of male twins	171	13.22 ± 0.09	125	13.57 ± 0.12	0.0221
Female twins	317	13.52 ± 0.07	278	14.02 ± 0.08	0.0001
DZ twins					
Spouses of male twins	107	13.29 ± 0.19	89	13.71 ± 0.14	0.0219
Female twins	233	13.59 ± 0.09	225	14.17 ± 0.10	0.0001

TABLE 57. Age at Marriage. Comparison of Younger (≤ 44 Years) and Older (> 44 Years) Female Twins and Spouses for the Mean Age at Marriage

Zygosity	Younger twins N	Younger twins Mean	Older twins N	Older twins Mean	Probability of t for older vs younger twins and spouses
MZ twins					
Male twins	211	25.37 ± 0.25	177	27.59 ± 0.38	0.0001
Spouses of male twins	195	22.24 ± 0.24	170	23.96 ± 0.31	0.0001
Female twins	284	22.59 ± 0.20	251	25.02 ± 0.26	0.0001
Spouses of female twins	239	25.25 ± 0.30	165	27.76 ± 0.38	0.0001
DZ twins					
Male twins	115	24.92 ± 0.39	125	28.80 ± 0.49	0.0001
Spouses of male twins	111	22.81 ± 0.35	113	23.69 ± 0.31	0.0691
Female twins	204	23.07 ± 0.24	203	25.19 ± 0.30	0.0001
Spouses of female twins	170	25.95 ± 0.36	137	28.71 ± 0.45	0.0001

TABLE 58. Parental Age at First Birth. Comparison of Younger (≤ 44 Years) and Older (> 44 Years) Twins and Spouses for the Mean Parental Age at First Birth

Zygosity	Younger twins N	Younger twins Mean	Older twins N	Older twins Mean	Probability of t for younger vs older twins and spouses
MZ twins					
Male twins	182	25.91 ± 0.28	132	28.66 ± 0.47	0.0001
Spouses of male twins	180	23.17 ± 0.27	129	25.43 ± 0.39	0.0001
Female twins	259	23.41 ± 0.24	222	26.02 ± 0.28	0.0001
Spouses of female twins	218	26.45 ± 0.32	141	28.94 ± 0.42	0.0001
DZ twins					
Male twins	111	25.98 ± 0.40	112	29.21 ± 0.38	0.0001
Spouses of male twins	109	23.89 ± 0.40	110	25.01 ± 0.38	0.0347
Female twins	196	23.73 ± 0.26	194	26.09 ± 0.31	0.0001
Spouses of female twins	163	26.91 ± 0.37	127	29.57 ± 0.45	0.0001

TABLE 59. Interval Between Marriage and First Birth. Comparison of Younger (≤ 44 Years) and Older (> 44 Years) Male and Female Twins for the Mean Interval Between Marriage and First Birth

Zygosity	Younger twins N	Younger twins Mean	Older twins N	Older twins Mean	Probability of t for older vs younger twins
MZ twins					
Male twins	171	1.15 ± 0.11	128	1.54 ± 0.18	0.0629
Female twins	235	1.33 ± 0.11	198	1.54 ± 0.26	0.2521
DZ twins					
Male twins	101	1.49 ± 0.15	108	1.50 ± 0.16	0.9821
Female twins	167	1.44 ± 0.13	172	1.56 ± 0.14	0.5101

TABLE 60. Comparison of Younger (≤ 44 Years) and Older (> 44 Years) Twins for the Number of Total Pregnancies, Unaffected Pregnancies, and First-Trimester Spontaneous Abortions

Zygosity	Younger twins N	Younger twins Mean	Older twins N	Older twins Mean	Probability of t for older vs younger twins
Total pregnancies					
MZ males	236	2.26 ± 0.10	202	2.82 ± 0.12	0.0003
MZ females	324	2.51 ± 0.08	300	2.79 ± 0.11	0.0408
DZ males	136	2.26 ± 0.11	150	2.39 ± 0.14	0.4500
DZ females	240	2.45 ± 0.10	242	2.63 ± 0.11	0.2083
Unaffected pregnancies					
MZ males	236	2.06 ± 0.08	202	2.62 ± 0.11	0.0001
MZ females	324	2.34 ± 0.08	300	2.58 ± 0.10	0.0595
DZ males	136	2.05 ± 0.10	150	2.21 ± 0.13	0.3365
DZ females	240	2.23 ± 0.09	242	2.41 ± 0.09	0.1665
First-trimester spontaneous abortions					
MZ males	236	0.203 ± 0.03	202	0.193 ± 0.04	0.8386
MZ females	324	0.167 ± 0.03	300	0.210 ± 0.03	0.2660
DZ males	136	0.215 ± 0.04	150	0.187 ± 0.06	0.7820
DZ females	240	0.217 ± 0.04	242	0.219 ± 0.03	0.9618

TABLE 61. Age of Menarche. Comparison of Unaffected and Affected Female Twins and Spouses of Male Twins for Mean Age of Menarche

Zygosity	Affected twins N	Affected twins Mean	Unaffected twins N	Unaffected twins Mean	Probability of t for affected vs unaffected twins
MZ twins					
Spouses of male twins	49	13.20 ± 0.20	247	13.39 ± 0.08	0.3470
Female twins	88	13.52 ± 0.12	507	13.79 ± 0.06	0.0418
DZ twins					
Spouses of male twins	39	13.54 ± 0.20	157	13.47 ± 0.10	0.7643
Female twins	78	13.73 ± 0.15	380	13.91 ± 0.07	0.3002

in unaffected pregnancies between the younger and older twins. No significant difference between the two age groups was seen in any category for first-trimester spontaneous abortions.

4.3.7. Comparison of families with and without a spontaneous abortion. Tables 61 through 66 give the results of comparing twins who had had one or more first-trimester spontaneous abortions (designated "affected") with those who had not (designated "unaffected"). The age of menarche (Table 61) was significantly lower in MZ females in the affected group ($P = 0.0418$). This was not repeated in any other category. The age of marriage (Table 62) was significantly different between affected and unaffected groups only for DZ male twins, where the affected group had a lower age at marriage ($P = 0.0397$). Only MZ male twins had a significant difference between the affected and unaffected groups for the age at the first birth (Table 63). MZ male twins also had a significantly shorter interval between marriage and the first birth

TABLE 62. Age at Marriage. Comparison of Unaffected and Affected Twins and Spouses for the Mean Age at Marriage

	Affected		Unaffected		Probability of t for affected vs unaffected twins and spouses
Zygosity	N	Mean	N	Mean	
MZ twins					
Male twins	63	25.87 ± 0.55	325	26.49 ± 0.25	0.3067
Spouses of male twins	62	23.32 ± 0.52	303	22.99 ± 0.22	0.5275
Female twins	88	24.22 ± 0.51	447	23.60 ± 0.17	0.2608
Spouses of female twins	65	26.34 ± 0.49	339	26.19 ± 0.27	0.7835
DZ twins					
Male twins	43	25.77 ± 0.57	197	27.19 ± 0.37	0.0397
Spouses of male twins	42	23.12 ± 0.53	182	23.31 ± 0.26	0.7477
Female twins	78	23.99 ± 0.47	329	24.16 ± 0.22	0.7289
Spouses of female twins	60	27.65 ± 0.68	247	27.07 ± 0.32	0.4305

TABLE 63. Parental Age at First Birth. Comparison of Affected and Unaffected Twins and Spouses for the Mean Parental Age at First Birth

	Affected		Unaffected		Probability of t for affected vs unaffected twins and spouses
Zygosity	N	Mean	N	Mean	
MZ twins					
Male twins	50	25.86 ± 0.61	264	27.29 ± 0.29	0.0464
Spouses of male twins	48	23.63 ± 0.59	261	24.19 ± 0.26	0.3804
Female twins	78	24.83 ± 0.43	403	24.55 ± 0.21	0.5943
Spouses of female twins	57	27.46 ± 0.56	302	27.39 ± 0.35	0.9175
DZ twins					
Male twins	43	26.86 ± 0.68	180	27.78 ± 0.33	0.2208
Spouses of male twins	42	24.16 ± 0.61	177	24.51 ± 0.30	0.6016
Female twins	77	24.69 ± 0.58	313	24.96 ± 0.27	0.6075
Spouses of female twins	60	28.68 ± 0.66	230	27.71 ± 0.33	0.2977

TABLE 64. Interval Between Marriage and First Birth. Comparison of Unaffected and Affected Twins for Mean Interval Between Marriage and First Birth

Zygosity	Affected N	Affected Mean	Unaffected N	Unaffected Mean	Probability of t for affected vs unaffected twins
MZ twins					
Male twins	44	0.93 ± 0.18	255	1.38 ± 0.11	0.0345
Female twins	71	1.37 ± 0.17	362	1.49 ± 0.15	0.5655
DZ twins					
Male twins	39	1.62 ± 0.34	170	1.47 ± 0.11	0.6888
Female twins	69	1.38 ± 0.23	270	1.54 ± 0.11	0.4923

TABLE 65. Present Age. Comparison of Unaffected and Affected Twins and Spouses for Mean Present Age

Zygosity	Affected N	Affected Mean	Unaffected N	Unaffected Mean	Probability of t for affected vs unaffected twins and spouses
MZ twins					
Male twins	67	44.82 ± 1.10	341	45.43 ± 0.50	0.6180
Spouses of male twins	63	42.35 ± 1.20	307	42.19 ± 0.54	0.9071
Female twins	93	48.09 ± 1.06	489	46.58 ± 0.44	0.1212
Spouses of female twins	67	49.29 ± 1.28	346	47.91 ± 0.53	0.2959
DZ twins					
Male twins	44	44.29 ± 1.21	206	46.68 ± 0.61	0.0974
Spouses of male twins	43	41.65 ± 1.31	186	42.97 ± 0.62	0.3581
Female twins	82	46.82 ± 1.05	354	46.62 ± 0.48	0.8506
Spouses of female twins	61	49.77 ± 1.24	249	48.82 ± 0.64	0.5071

TABLE 66. Comparison of Unaffected and Affected Twins for the Number of Total Pregnancies and Unaffected Pregnancies

Zygosity	Affected N	Affected Mean	Unaffected N	Unaffected Mean	Probability of t for affected vs unaffected twins
Total pregnancies					
MZ males	67	4.27 ± 0.18	371	2.20 ± 0.08	0.0001
MZ females	92	4.17 ± 0.20	532	2.37 ± 0.07	0.0001
DZ males	44	4.00 ± 0.19	242	2.02 ± 0.09	0.0001
DZ females	82	4.09 ± 0.16	400	2.23 ± 0.07	0.0001
Unaffected pregnancies					
MZ males	67	2.97 ± 0.16	371	2.20 ± 0.08	0.0001
MZ females	92	2.90 ± 0.18	532	2.37 ± 0.07	0.0037
DZ males	44	2.73 ± 0.19	242	2.02 ± 0.09	0.0017
DZ females	82	2.80 ± 0.15	400	2.23 ± 0.07	0.0008

TABLE 67. Spearman Correlations Between MZ and DZ Twins for the Number of Total Pregnancies, Unaffected Pregnancies, and First-Trimester Spontaneous Abortions

	MZ twins		DZ twins	
	Males	Females	Males	Females
Total pregnancies				
r	0.31286	0.27979	0.15449	0.11752
P	0.0001	0.0001	0.0654	0.0686
N	219	312	143	241
Unaffected pregnancies				
r	0.35152	0.31638	0.12073	0.09140
P	0.0001	0.0001	0.1509	0.1572
N	219	312	143	241
First-trimester spontaneous abortions				
r	0.00614	0.15432	−0.01956	0.22966
P	0.9281	0.0061	0.8166	0.0022
N	219	312	143	241

in the affected group (Table 64). There was no significant difference for the present age of the twin in any category between affected and unaffected groups (Table 65). There was a significant difference between the groups for the total number of pregnancies reported and for the number of unaffected pregnancies (Table 66). For both MZ and DZ twins, the affected groups had more total pregnancies and more unaffected pregnancies.

Table 67 gives the Spearman correlation coefficients of twins with respect to total pregnancies and first-trimester spontaneous abortion. Both male and female MZ twins had correlation coefficients significantly greater than zero for total pregnancies (0.31286 and 0.27979, respectively), as well as for unaffected pregnancies (0.35152 and 0.31638, respectively). DZ twins were not significantly correlated for number of total pregnancies or for unaffected pregnancies. However, both MZ and DZ female twins were significantly correlated for first-trimester spontaneous abortions, whereas MZ and DZ male twins were not.

5. DISCUSSION

The zygosity questionnaire (Q1) proved to be a valid indicator of the zygosity status as determined by a blood typing study. The percentage agreement between results of the zygosity questionnaire and those obtained from blood typing was high, nearly 98%; however, this also means that as many as 37 twin individuals may have been misclassified in this sample of 1,830. Misclassification results in a decrease in the variance within DZ pairs and an increase in the variance within MZ pairs, thus making genetic

effects more difficult to detect. Positive findings in a questionnaire study would probably be conservative estimates of the genetic influence on a given trait. The zygosity questionnaire was a time- and cost-effective way to classify large numbers of twins.

The incidence of total spontaneous abortions (both early and late) was between 8% and 9%; this was low compared to the frequently quoted incidence of 10–15%, but may be explained by the tendency to underestimate the occurrence of spontaneous abortion when data are recalled from the memory of the respondent. This is particularly true when the respondent is over 50 years of age [85]. Recall may represent a major problem in analyses of the frequency of occurrence of spontaneous abortion; those females with more than one spontaneous abortion or those whose cotwin or sister-in-law had also had a spontaneous abortion may recall the incident more reliably than would the female with an isolated case of fetal loss. No difference was found in the mean number of spontaneous abortions between those twins born between 1915 and 1935 ("older") and those born between 1936 and 1946 ("younger"). Although the youngest of twins may not have finished producing offspring, any secular trends influencing the frequency of spontaneous abortions would be expected to change the mean number of spontaneous abortions. The inclusion of induced abortion would tend to inflate the calculated frequency of spontaneous abortion. A questionnaire study, relying on volunteered information, cannot of itself discriminate between spontaneous and induced abortion. Some women may have been reluctant to admit that an abortion was induced; however, since the overall frequency of spontaneous abortion did not appear to be inflated, the inclusion of a large number of induced abortions was probably not a significant problem.

The frequency of twin births reported by this sample population was approximately 1.06%, which is lower than the mean twinning rate, 1.22%, for the Norwegian population during the time period studied (see Appendixes 2 and 3). The incidence of twin births was highest among the DZ female twins and lowest among the MZ male twins. Considering the known genetic influence on DZ twinning, it was not surprising that this study also showed that DZ twins had a significantly higher incidence of twin births than did MZ twins. Overall, females had a significantly higher incidence of twin births, perhaps reflecting the sex-limited nature of the tendency for DZ twinning.

Marital status was a variable that appeared to be influenced by zygosity and by the interaction of sex within zygosity. MZ twins, both male and female, had more concordant pairs for "never married" and fewer pairs discordant for marital status than was expected based on random distribution of marital status. Among DZ twins, only males differed significantly from expected. The overall heterogeneity χ^2 substantiated the differences in zygosity and sex. It was apparent from this analysis that zygosity alone contributed significantly to the heterogeneity, whereas sex did not. The significant sex-by-zygosity interaction reflects the different results obtained when comparing males and females within zygosity.

Case ascertainment is one of the more challenging aspects of population studies. A considerable amount of time, effort, and money must be spent to obtain samples of sufficient size. In this study, addresses were found for and initial questionnaires were sent to 2,365 pairs of same-sexed twins, and of these only 915 pairs (38%) provided data sufficient for analysis. This might, however, be considered to be only the initial phase of the collaborative Norwegian-Virginian twin project. An ever-increasing number of same-sexed twins are easily accessible for special studies, and there are enormous amounts of data now available for analysis. One of the advantages of a popula-

tion-based registry is the minimizing of volunteer bias [96]; however, an investigation of the response rate by sex and zygosity is warranted as it is likely that there are differences in accuracy and motivation between sexes, between zygosities, and between twins and nontwins.

Maternal effects on spontaneous abortion were shown clearly in a sample size of 428 pairs of MZ twins. In a model using the entire sample of twins and with the combined parameter c functionally dependent on parameters a and b, the maximum likelihood estimate was strikingly higher for the maternal risk than for the parental risk. As might be expected, the removal of families with "habitual abortions" lowered the estimate of the maternal risk; however, the decrease was minimal. These data indicate that, since the maternal risk estimates were similar whether or not "habitual aborters" were included in the analysis, the recurrence risk was not the exclusive burden of the habitual aborter. By fixing the recurrence risk parameters (a, b, and c) and the population frequency (p) to zero, it was possible to make likelihood estimates using a model in which all cases of first-trimester spontaneous abortion were assumed to be sporadic in their occurrence. The difference of the two log likelihoods indicated that the model allowing for only a sporadic etiology of early fetal loss had a significantly poorer fit than the model allowing for recurrence risks. Similarly, a model in which the maternal and paternal risks were forced to be equal had a significantly poorer fit than the model allowing the risks to be unequal. There was no indication of heterogeneity in the estimation of the parameters when twins over the age of 44 and those 44 years of age or younger were analyzed separately. The similarity of findings in the two age groups thus gives no hint that there have been major secular trends in the etiology of early fetal loss, and provides reassuring evidence that the questionnaire responses from older women are not biased in a systematic manner.

The population frequency of individuals at high risk for having a spontaneous abortion was estimated to be over 55%. This may seem to be quite high, but for a trait as frequently occurring and as heterogeneous as early fetal loss, it is not unexpected. The low estimate of the sporadic risk was somewhat unexpected, though, and it may indicate that the majority of causes of early spontaneous abortion carry a substantial recurrence risk. Many of the known or suspected causes of spontaneous abortion may have detectable recurrence risks. For example, over half of early spontaneous abortions are due to chromosome abnormalities, and over half of the chromosome abnormalities involve nondisjunction. As there is some evidence that nondisjunction may be under genetic control, this could contribute substantially to the recurrence risk. Balanced translocation heterozygotes have a demonstrable recurrence risk, although their relative rarity may make their contribution to the overall recurrence risk slight. Other causes such as ABO incompatibility or physical abnormalities of the reproductive tract clearly carry recurrence risks. All of these various causes appear to add to the maternal recurrence risk of about 13%.

Although many of the traits that can cause spontaneous abortion may reside in the male parent, they may not contribute to fetal loss. For example, hyperparathyroidism in the male parent does not appear to increase the risk of spontaneous abortion. The unique and intimate relationship of the female parent and fetus allow conditions and exposures in the mother to affect the fetus. Chromosome abnormalities may occur in the germ cells of either parent, but owing to differences in the life cycle of ova compared to spermatozoa, a random abnormality is more likely to be transmitted to the offspring if it occurs in a female than in a male germ cell. Immunologic factors such

as blood groups and HLA would be an example of a burden shared by both parents. Only rarely might a cause of early fetal loss be classified as exclusively paternal, as in the case of a paternally carried balanced translocation.

The results of the MZ half-sib study for the maternal effect on spontaneous abortion yielded reasonable estimates of both the recurrence risk and the frequency of high-risk individuals. Based on the recurrence risk estimates given by Lauritsen [80], a weighted mean recurrence risk of 18.8% would be expected in a sample consisting of women with an index abortion, assuming that approximately 60% of the abortuses would be chromosomally abnormal and 40% chromosomally normal (see paragraph 2.3). One would expect a lower recurrence risk in a sample among whom not all of the women have had an index abortion. An overall recurrence risk of 13% seems reasonable when the sample population is a combination of etiologies.

When the various factors related to biologic reproduction are considered, one would predict a strong maternal effect in the occurrence of spontaneous abortion. In the present study, this hypothesis was tested and substantiated: Maternal factors were a major determinant of the risk of first-trimester spontaneous abortion.

The results of analyzing the age of menarche in several ways all substantiated the previous findings of genetic control over the onset of menstruation [125,159]. In this study, the correlation between MZ female twins was about 0.70, and about 0.29 for DZ female twins. Previous studies have reported correlations as high as 0.93 and 0.62 for MZ and DZ female twin pairs [42]. A significantly smaller mean difference in the age of menarche was found for MZ female twins than for DZ female twins, and the within-pair variance for MZ twins was significantly less than that for DZ twins. The model-fitting procedure used indicated that in the total sample of MZ and DZ female twins, additive genetic, V_A, and a within-pair environmental component, V_{EW}, could account for the variation seen in the age of menarche. This did not remain consistent when the twins were divided on the basis of present age. In the younger twins, the within-pair variance for DZ pairs increased considerably compared with the within-pair variance in older DZ pairs, and the simple V_A, V_{EW} model fit only the data for the older twins. Resolution of this contradiction was accomplished by adding an additional environmental component for the different time strata, V_{ES}.

It has been estimated that the mean age of menarche has decreased over the past century [156]. Very little change in the mean variability has been reported, however, and the standard deviation has remained at approximately 1 year [157]. In Norway, the mean age of menarche in 1869 was approximately 16 years of age, whereas in 1970 it was approximately 13.25 years of age. This decrease has apparently slowed or halted since 1950 [18] and there is some indication that the estimate of a mean age of 16 years of age is inflated since it was based on a very small sample size [18a]. A significant decrease in the age of menarche was also seen in this population, with the younger females having a mean age of menarche of 13.41 years and the older females a mean age of 13.87 years. As noted earlier, an analysis of variance showed a marked difference in the variation within DZ pairs between older and younger twins. It is possible that the environment of females born between 1915 and 1935 was different from the environment of females born between 1936 and 1946, and that the "younger" environment resulted in an increase in the phenotypic variance within DZ pairs. For example, it is known that severe nutritional deprivation can delay menarche. Assuming that this would affect both twins, it may increase their similarity in the onset of menstruation. When the environment changes and nutritional deprivation is no longer a factor, the onset of menstruation becomes more varied, based more on the genotype of the

individual twin. As illustrated by the model-fitting procedure and the analysis of variance, it does appear that the environments of the younger and older twins are contributing to the heterogeneity of the total sample, and that the environment of the younger twins increases the difference within DZ pairs.

The age of menarche must be examined with caution, as inaccuracies in recall have been established. A prospective Swedish study of 339 schoolgirls noted that the correlation between the actual and recalled age of menarche was 0.81 ± 0.05 four years past the actual onset of menstruation [10]. Approximately 63% of the women recalled the date of menarche to within 3 months. In this study, the age of menarche was rounded to the nearest whole year, which certainly must contribute to inaccuracy.

One interesting finding was that female twins, both MZ and DZ, tended to have a later age at onset of menstruation than nontwin females. This may be related to the biology of twinning itself or perhaps to a delayed effect of the tendency for twins to have lower birthweights. However, it should be noted that the mean present age of the spouses of male twins was significantly lower than that of female twins (42.22 ± 0.49 and 46.90 ± 0.39 for spouses of MZ males and MZ female twins, respectively, and 42.73 ± 0.56 and 46.76 ± 0.42 for the spouses of DZ males and DZ females, respectively). It may be that the lower age of the spouses of twins could put them more into the age group with an earlier age of menarche.

The mean difference in the age of menopause was found to be significantly less in female MZ twins than in DZ female twins, although an F-test of the within-pair variance was not significant at the 0.05 level. A weighted least-squares analysis on the age of menopause indicated that a model with the additive genetic component and the within-pair environmental component fit the data quite well. Age of menopause is considerably more variable than the age of menarche, particularly since its onset is not clearly demarcated. Studies of the genetic control of menopause often use different criteria to define the cessation of menstruation, and it is difficult to assess the secular changes in the mean age of menopause. Some studies have noted that the mean age of menopause appears to be increasing in developed countries [6]. This sample size was quite small, increasing the unreliability of the data.

The female spouses of male DZ twins were significantly more similar for the age of marriage than the male spouses of female DZ twins. The same trend was found in MZ twins, but it was not significant. One possible explanation may be that male twins tend to marry women closer in age to each other than female twins do. All of the correlations, however, were significant, indicating that all people tend to marry people about the same age as themselves. The mean age of marriage decreased significantly between the two age groups, falling from about 28 years of age to 25 years in males and from about 24½ years of age to 22 years in females. This decline was also reflected in the decrease in the age at first birth, as the interval between marriage and first birth remained the same. The age at first birth was more similar in female than in the male MZ twins. No significant difference between MZ and DZ twins was seen for the mean difference in the age at first birth. Female MZ twins had a correlation coefficient of 0.42, whereas males had only a 0.25 correlation. The DZ twins were the opposite of the MZ twins, with the DZ males having a correlation coefficient of 0.41 and the females only 0.29. All of the correlations were significant, however. The same pattern was seen in the interval between marriage and first birth; the only significant correlations were found between MZ female twins and DZ male pairs. The mean interval and mean difference in interval did not differ significantly in any comparison.

Environmental changes can result in differences between older and younger twins. As noted earlier, the age of menarche had decreased significantly, as had the age of marriage and the age at first birth. It is possible that environmental influences have changed over the course of the 32 years studied. The years in the 1935–1945 period, during which most of the twins born between 1915 and 1925 would be "marriageable," were years of depression and war, including the occupation of Norway. This may have delayed marriage and, subsequently, the birth of the first child. The number of pregnancies not ending in early spontaneous abortion, which approximates family size, had decreased when older and younger twins were compared, but the difference was significant only in MZ male twins. The younger twins' present age ranged from 34 to 44 years and the youngest of these may not have completed their families. No significant difference, however, was found for the mean number of early spontaneous abortions.

Differences between the group of twins reporting at least one spontaneous abortion and the group not reporting any were examined for age of menarche, marriage, first birth, interval between marriage and first birth, and total and unaffected pregnancy number. Only MZ female twins were significantly different in the mean age of menarche with those reporting a spontaneous abortion having a lower age of menarche. Age at marriage showed no consistent trend, although DZ males reporting a spontaneous abortion were found to be significantly younger than those without spontaneous abortion. For age at first birth, no consistent trend was found, although, again, the MZ male twins with a spontaneous abortion were significantly younger at the first birth than those without a spontaneous abortion. The MZ males also had a significantly lower interval between marriage and first birth, but this was not found in any of the other groups. Because of the lack of consistency, it is concluded that these variables are probably not influenced by the presence or absence of a spontaneous abortion. There was also no evidence of a difference in the present age between twins reporting a spontaneous abortion and those not, which was another way of looking for secular trends. There was a significant difference in all groups for the number of total pregnancies, with the twins reporting a spontaneous abortion having the larger number of total pregnancies. This is evidence for the compensation phenomena following early fetal loss [168]; however, there appeared to be some degree of over-compensation reflected in the significantly larger number of unaffected pregnancies in the groups reporting a spontaneous abortion. A co-twin control analysis supported the overcompensation hypothesis. In 213 pairs of whom one twin reported a spontaneous abortion and the cotwin did not, the twin with a spontaneous abortion had significantly more unaffected pregnancies than the cotwin. It is unlikely that this represents an effect of gravidity. As mentioned previously, the effect of gravidity has had inconsistent assessments, and the increase in the number of pregnancies has been thought to be due to compensation for the loss rather than a true effect of gravidity [73]. The population genetics implication of over-compensation would be an increase in the number of high-risk individuals over time if the trait were genetically determined. The half-sib analysis for spontaneous abortion did show, among the MZ twins, a small but nonsignificant increase in the estimate of the population frequency in younger twins as compared to older twins (56% and 53%, respectively); however, the incidence of spontaneous abortion was not significantly different in the two age groups. Additionally, the correlation coefficients of MZ and DZ female twins for first-trimester spontaneous abortion pro-

vided no hint that the tendency for abortion has a strong genetic effect; indeed, the correlation coefficient was higher in DZ females.

The total number of pregnancies and the number of unaffected pregnancies were significantly correlated for MZ twins, both male and female, but were not significantly correlated for DZ twins. This would seem to indicate a tendency for MZ twins to coordinate their family size, whereas DZ twins would not be so inclined. However, both MZ and DZ females were significantly correlated for the number of spontaneous abortions, whereas neither MZ nor DZ males were. This seems to reiterate the strong maternal effect on spontaneous abortion.

6. CONCLUSIONS

Analysis of spontaneous abortion data using the half-sib model for qualitative traits indicated the presence of a strong maternal effect and suggested that over 54% of all women may belong to a high risk group; these high risk women then have approximately a 13% risk for early fetal loss. The prediction of a significant maternal effect in spontaneous abortion based on biological considerations was substantiated using this new statistical method.

The age of menarche was shown to be under significant genetic control based on the comparison of MZ and DZ female twins. In addition, an interesting environmental effect was found; the environmental effect on the age of menarche appeared to differ between DZ twins born in 1915–1935 and those born in 1935–1946. Several other secular changes were noted including a decrease in the mean age of menarche and in the mean age at marriage and birth of the first child. No secular differences were found for the mean interval between marriage and the birth of the first child nor in the mean number of early spontaneous abortions. Overcompensation for fetal loss was indicated by the fact that twins reporting a spontaneous abortion had more unaffected pregnancies than twins not reporting a spontaneous abortion.

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APPENDIX 1. Summary of Twin Registry Results by Year, 1915–1946

Category	1915	1916	1917	1918	1919	1920	1921
Total twin pairs	774	810	893	820	743	953	801
Opposite-sexed	267	301	327	300	271	332	322
Same-sexed females	239	220	256	281	226	279	229
Same-sexed males	268	289	310	239	246	342	250
Same-sexed total	507	509	566	520	472	621	479
Not found	105	126	120	109	79	106	96
1 or 2 stillborn	39	43	51	66	12	90	3
1 or 2 early death	108	97	114	79	28	68	110
Previously used	27	24	24	23	22	35	23
Total found	228	219	257	243	223	322	247
Complete yes	54	61	62	74	79	90	67
Complete no	10	11	16	21	12	13	24
Incomplete	44	53	56	37	28	61	41
1 stillborn or early death	30	27	27	25	38	55	30
1 late death	25	21	16	26	20	32	18
Not a twin	4	1	5	11	8	11	2
Returned blank	14	17	16	17	4	13	5
No response	47	28	59	32	34	47	60

Category	1922	1923	1924	1925	1926	1927	1928
Total twin pairs	859	840	738	710	778	686	680
Opposite-sexed	364	312	283	242	298	252	240
Same-sexed females	250	254	239	262	246	207	290
Same-sexed males	245	274	216	206	234	227	231
Same-sexed total	495	528	455	468	480	424	521
Not found	118	116	88	79	63	56	84
1 or 2 stillborn	43	57	48	56	60	42	54
1 or 2 early death	84	88	54	43	44	59	33
Previously used	23	16	13	24	9	5	13
Total found	227	251	252	266	293	267	239
Complete yes	64	83	79	69	29	21	43
Complete no	16	14	16	11	1	5	3
Incomplete	40	48	61	74	44	50	62
1 stillborn or early death	32	24	30	28	24	17	18
1 late death	16	11	8	9	7	11	6
Not a twin	1	3	1	1	0	1	2
Returned blank	7	8	4	6	0	0	0
No response	51	60	53	68	188	162	105

Category	1929	1930	1931	1932	1933	1934	1935
Total twin pairs	714	679	553	610	566	521	534
Opposite-sexed	256	260	200	216	225	183	208
Same-sexed females	222	187	166	170	151	166	171
Same-sexed males	236	232	187	224	190	172	155
Same-sexed total	458	419	353	394	341	338	326
Not found	83	60	34	26	27	36	36
1 or 2 stillborn	64	41	18	73	57	35	34
1 or 2 early death	34	37	38	46	33	26	32
Previously used	22	4	17	15	20	9	0

Appendix 1 continued next page.

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APPENDIX 1, continued. Summary of Twin Registry Results by Year, 1915–1946

Category	1929	1930	1931	1932	1933	1934	1935
Total found	252	259	246	234	204	232	216
Complete yes	45	60	75	72	75	85	73
Complete no	6	4	11	8	4	12	7
Incomplete	65	74	46	60	57	67	48
1 stillborn or early death	17	13	28	19	15	14	15
1 late death	2	4	6	16	3	4	6
Not a twin	1	4	7	4	3	2	3
Returned blank	0	0	9	7	5	6	7
No response	116	102	64	48	42	42	57

Category	1936	1937	1938	1939	1940	1941	1942
Total twin pairs	551	562	605	636	655	570	570
Opposite-sexed	210	228	217	221	237	194	173
Same-sexed females	174	157	173	209	195	168	207
Same-sexed males	167	177	215	206	223	208	190
Same-sexed total	341	334	388	415	418	376	397
Not found	45	30	39	33	32	41	36
1 or 2 stillborn	30	37	38	35	49	41	31
1 or 2 early death	31	29	32	34	48	31	42
Previously used	0	0	0	0	0	4	3
Total found	235	238	279	313	289	259	286
Complete yes	77	88	103	136	109	74	86
Complete no	10	14	10	6	18	7	5
Incomplete	48	51	66	65	48	72	78
1 stillborn or early death	15	16	17	15	29	15	9
1 late death	5	6	8	11	5	4	4
Not a twin	5	1	3	7	7	2	2
Returned blank	2	3	5	8	5	2	0
No response	73	59	67	65	68	83	102

Category	1943	1944	1945	1946
Total twin pairs	671	729	705	838
Opposite-sexed	242	222	252	314
Same-sexed females	192	234	212	254
Same-sexed males	237	273	241	270
Same-sexed total	429	507	453	524
Not found	35	28	45	9
1 or 2 stillborn	41	56	39	69
1 or 2 early death	36	41	57	65
Previously used	10	3	5	11
Total found	307	379	307	370
Complete yes	90	107	97	185
Complete no	5	4	6	16
Incomplete	95	112	92	84
1 stillborn or early death	10	9	12	24
Late death	3	5	1	7
Not a twin	5	1	1	9
Returned blank	1	1	3	6
No response	98	140	95	39

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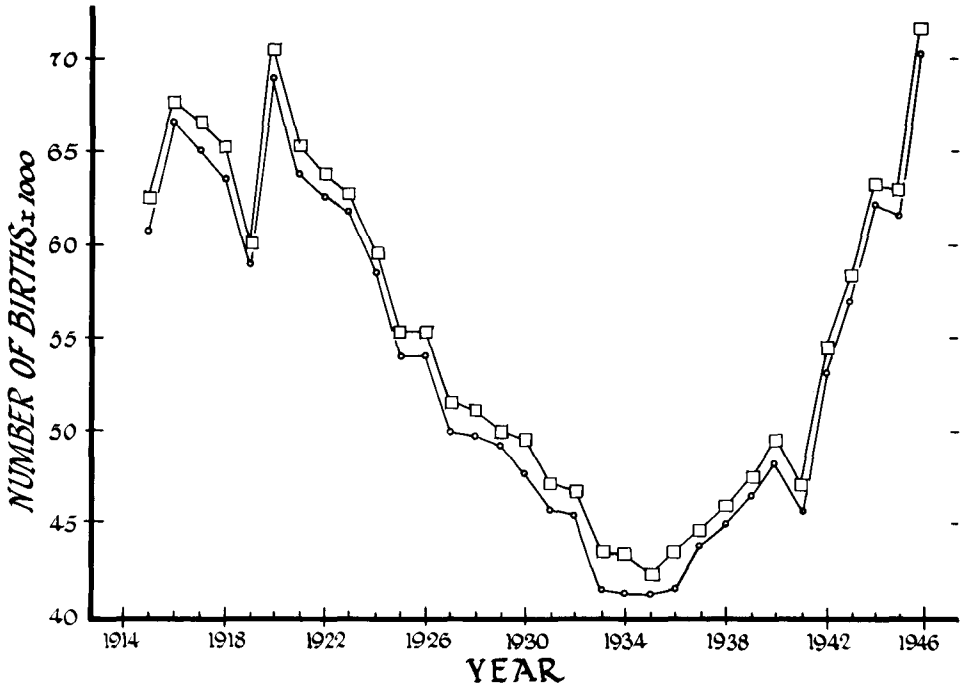
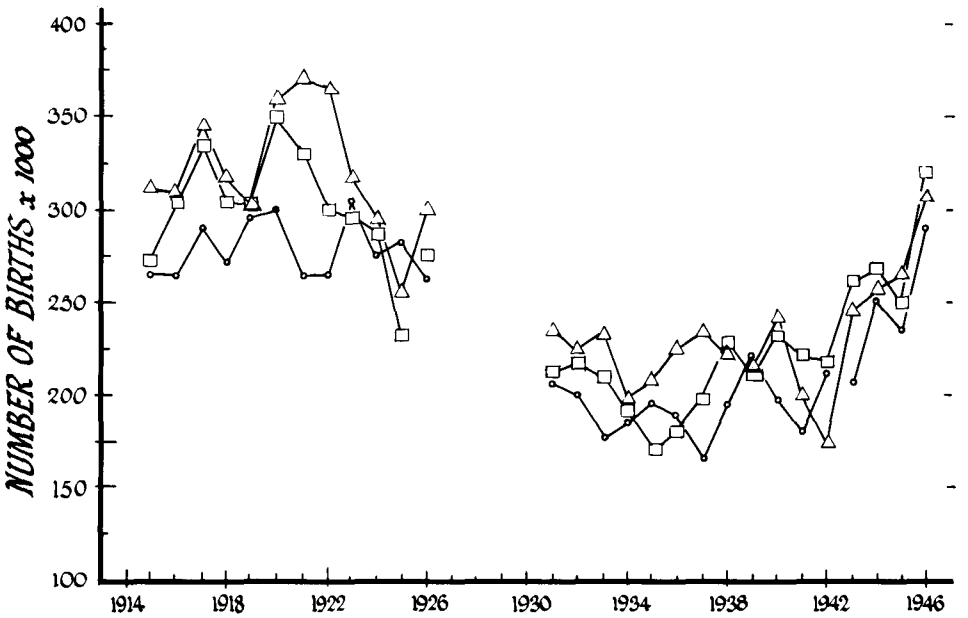


APPENDIX 2. Live-births (○) and Total Births (□) Recorded in the Norwegian Central Statistics Archives, 1915–1946



APPENDIX 3. Same-sexed Male (□), Same-Sexed Female (○), and Opposite-Sexed (△) Twin Pairs Recorded in the Norwegian Central Statistics Archives, 1915–1946 (Except for 1927–1930)

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*APPENDIX 4. Comparison of Norwegian Twin Registry (NTR) to Central Statistics (CS) for Total Number of Same-Sexed Twins Noted, Including Stillborns; 1915–1946**

Year	Source	Same-sex males		Same-sex females		Total same-sex	
		Total	% CS in NTR	Total	% CS in NTR	Total	% CS in NTR
	NTR	268		239		507	
1915**			0.98		0.89		0.93
	CS	274		270		544	
	NTR	289		220		509	
1916			0.95		0.81		0.89
	CS	302		269		571	
	NTR	310		256		566	
1917			0.91		0.89		0.90
	CS	338		288		626	
	NTR	239		281		520	
1918			0.79		1.04		0.91
	CS	302		269		571	
	NTR	246		226		472	
1919			0.84		0.78		0.81
	CS	294		291		585	
	NTR	342		279		621	
1920			0.99		0.95		0.97
	CS	347		293		640	
	NTR	250		229		479	
1921			0.77		0.85		0.81
	CS	323		269		592	
	NTR	245		250		495	
1922			0.84		0.93		0.88
	CS	292		268		560	
	NTR	274		254		528	
1923			0.94		0.87		0.91
	CS	290		292		582	
	NTR	216		239		455	
1924			0.76		0.87		0.82
	CS	284		274		558	
	NTR	206		262		468	
1925			0.88		0.93		0.91
	CS	235		281		516	
	NTR	234		246		480	
1926			0.86		0.96		0.91
	CS	271		256		527	
	NTR	187		166		353	
1931			0.86		0.80		0.83
	CS	217		206		423	
	NTR	224		170		394	
1932			1.02		0.89		0.97
	CS	220		190		410	
	NTR	190		151		341	
1933			0.90		0.88		0.89
	CS	212		172		384	
	NTR	172		166		338	
1934			0.92		0.92		0.92
	CS	186		180		366	

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*APPENDIX 4, continued. Comparison of Norwegian Twin Registry (NTR) to Central Statistics (CS) for Total Number of Same-Sexed Twins Noted, Including Stillborns; 1915–1946**

Year	Source	Same-sex males		Same-sex females		Total same-sex	
		Total	% CS in NTR	Total	% CS in NTR	Total	% CS in NTR
	NTR	155		171		326	
1935			0.91		0.91		0.91
	CS	171		187		358	
	NTR	167		174		341	
1936			0.93		0.94		0.94
	CS	179		185		364	
	NTR	177		157		334	
1937			0.90		0.96		0.93
	CS	196		163		359	
	NTR	215		173		388	
1938			0.95		0.90		0.92
	CS	227		193		420	
	NTR	206		209		415	
1939			0.95		0.95		0.95
	CS	216		219		435	
	NTR	223		195		418	
1940			0.96		0.99		0.98
	CS	232		196		428	
	NTR	208		168		376	
1941			0.95		0.94		0.95
	CS	219		178		397	
	NTR	190		207		397	
1942			0.88		0.98		0.93
	CS	217		211		428	
	NTR	237		192		429	
1943			0.94		0.95		0.94
	CS	253		203		456	
	NTR	273		234		516	
1944			1.07		0.95		1.02
	CS	255		247		502	
	NTR	241		212		453	
1945			0.98		0.93		0.96
	CS	245		227		472	
	NTR	270		254			
1946							
	CS	318		276			

*Excluding 1927–1930 where no details are available in the Central Statistics archives.
**Central Statistics data for 1915 averaged from 1911–1915.

APPENDIX 5. Zygosity Questionnaire (Q1)

Questionnaire for Twins

1. Name _____ Birthdate _____
   Address _____ Telephone _____
2. Is your twin alive? Check one. Yes ☐ No ☐
   If no, at what age did your twin die? _____
3. Name of your twin _____
   Address _____
4. Were you and your twin like two drops of water as you were growing up or were you just as alike as siblings? Check one.
   a. Like two drops of water ☐ b. Like siblings ☐ c. Don't know ☐
5. Did any of these people have trouble telling you and your twin apart when you were children? Check off how often.

	Always	Now and then	Never	Don't know
a. Parents	☐	☐	☐	☐
b. Siblings (if none, check here ☐)	☐	☐	☐	☐
c. Grandparents (if none, check here ☐)	☐	☐	☐	☐
d. Classmates	☐	☐	☐	☐
e. Teachers	☐	☐	☐	☐
f. Strangers	☐	☐	☐	☐

6. Mark with a check about you and your twin

	Exactly alike	Somewhat alike	Not alike	Don't know
a. Eye color	☐	☐	☐	☐
b. Hair color	☐	☐	☐	☐
c. Hair type	☐	☐	☐	☐
d. Height	☐	☐	☐	☐
e. Weight	☐	☐	☐	☐
f. Teeth	☐	☐	☐	☐
g. Voice	☐	☐	☐	☐
h. Musical ability	☐	☐	☐	☐
i. Handiness	☐	☐	☐	☐
j. Temperament	☐	☐	☐	☐
k. Musculature	☐	☐	☐	☐
l. Language ability	☐	☐	☐	☐

7. Do you and your twin have exactly the same tastes in the following things?

	Yes	No
a. Clothes	☐	☐
b. Food	☐	☐
c. Reading material	☐	☐
d. Films/TV	☐	☐
e. Music	☐	☐
f. Sports	☐	☐
g. Other free time activities	☐	☐

8. Do you yourself think that you and your twin are monozygotic or dizygotic?
   a. Monozygotic ☐ b. Dizygotic ☐ c. Don't know ☐

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APPENDIX 5. Zygosity Questionnaire (Q1) continued.

9. What reason do you have for your answer to question 8?
   a. We look alike ☐
   b. We don't look alike ☐
   c. My parents were told at our birth that we were monozygotic ☐
   d. My parents were told at our birth that we were dizygotic ☐
   e. There was only one placenta ☐
   f. There were two placentas
   g. We have the same blood type ☐
   h. We have different blood types
   i. Other reasons _____
10. Are there other twins in your family? Yes ☐ No ☐
    If yes, check off the relationship of the other twins to you:
    a. Mother ☐ b. Father ☐ c. Siblings ☐ d. Children ☐
    e. Other relations, please specify _____
11. Would you be willing to answer other questionnaires for this study? Yes ☐ No ☐

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