

Shared HLA Antigens and Reproductive Performance among Hutterites

C. L. OBER,¹ A. O. MARTIN,¹ J. L. SIMPSON,¹ W. W. HAUCK,² D. B. AMOS,³
D. D. KOSTYU,³ M. FOTINO,⁴ AND F. H. ALLEN, JR.⁴

SUMMARY

Shared histocompatibility antigens between spouses may affect reproductive outcome adversely as a result of prenatal selection against compatible fetuses. Evidence from both animal and human studies suggest that histocompatible fetuses may not initiate a maternal immunologic response that prevents rejection of the embryo. Therefore, parents sharing HLA antigens may produce compatible fetuses and consequently experience a greater frequency of early fetal losses and show poorer reproductive outcome than couples not sharing antigens. In the Hutterites, an inbred human isolate that proscribes contraception, we tested the hypothesis that couples sharing HLA antigens have poorer reproductive outcomes than couples who do not. The Hutterites are characterized by high fertility and large family sizes. Couples that share zero (no. = 21), one (no. = 15), and more than one (no. = 10) HLA-A or HLA-B antigens were compared for reproductive performance. Median intervals between births were larger among couples that share more than one antigen in eight of 11 intervals examined. In addition, the median intervals from marriage to first, fifth, and tenth birth were consistently larger among couples that share more than one antigen. Differences among the groups appear to become larger with increasing parity, suggesting that the effect of histocompatibility on reproductive performance becomes more evident in later pregnancies. These differences in reproductive performance between couples that share zero, one, or more than one HLA-A or HLA-B antigens may have significant evolutionary conse-

Received December 27, 1982; revised February 10, 1983.

This study was supported in part by grants N01-HD-0-2840, R01-CA-19822, and GM-10356, and Biomedical Sciences Support Grant 5-S05 RR07028 from the National Institutes of Health, and Biomedical Research Support Grant from the Dean's Office, Northwestern University Medical School.

¹ Section of Human Genetics, Department of Obstetrics and Gynecology, Northwestern University Medical School, 333 E. Superior Street, Chicago, IL 60611.

² Northwestern University Cancer Center and Department of Community Health and Preventive Medicine, Northwestern University Medical School, Chicago, IL 60611.

³ Division of Immunology, Duke University Medical School, Durham, NC 27710.

⁴ The New York Blood Center, 310 East 67th Street, New York, NY 10021.

© 1983 by the American Society of Human Genetics. All rights reserved. 0002-9297/83/3505-0019\$02.00

quences. However, our results demonstrate that sharing HLA antigens does not preclude normal pregnancy and caution should be exercised before concluding that shared HLA antigens are solely responsible for repeated fetal losses.

INTRODUCTION

Histocompatibility antigens common to both mother and fetus have been postulated to affect reproductive outcome adversely. The mechanism presumably reflects observations that maternal-fetal histoincompatibility stimulates a maternal immunologic response that enhances implantation and thus facilitates maintenance of pregnancy [1–3]. This maternal immunologic response may not be elicited if mother and fetus are histocompatible. Support for this hypothesis includes: (1) increased placental sizes in murine fetuses in which paternally derived histocompatibility antigens differ from maternal antigens [4, 5] and (2) higher implantation frequencies in histoincompatible murine zygotes than in histocompatible murine zygotes [6].

If histocompatible human fetuses are at a selective disadvantage during early pregnancy, couples sharing HLA antigens should have poorer reproductive outcomes than couples not sharing antigens, because in some offspring of the former both mother and fetus may share antigens. Indeed, couples experiencing repetitive spontaneous abortions in early gestation are reported to share more HLA antigens than do control couples [7–10]. However, these studies suffer from certain limitations inherent in retrospective studies as well as from inadequate controls. Examining this relationship in a sample not selected on the basis of reproductive histories would seem necessary to confirm the effect of sharing HLA antigens on reproductive performance and to permit derivation of models for selection at these loci. As a first step in this direction, we investigated the relationship between shared HLA-A and HLA-B antigens and reproductive outcome in a fertile population not selected on the basis of reproductive histories or HLA types. This opportunity arose during the course of ongoing investigations of the Hutterites [11–13], a population we have been studying for many years [14].

MATERIALS AND METHODS

Population Surveyed

The Hutterites, an Anabaptist religious isolate, live on colonies (communal farms) composed of 100–150 people. Presently, there are more than 200 colonies distributed in the northern United States and western Canada. Each colony belongs to one of three major subdivisions (S-, L-, or D-leut). Marriages are leut-endogamous, and relatively few Hutterites have joined or left the community since its members initially migrated to the United States from Russia in the 1870s [14–17]. Inbreeding among Hutterites results in many couples sharing HLA antigens, thus facilitating this study.

Of importance for the current study are several characteristics of Hutterite lifestyle. In particular, Hutterites prohibit contraception. Not surprisingly, the average completed

family size is large, 11.2 among S-leut [15] and 8.8 among L-leut [18]. In a study of the effects of inbreeding among S-leut, Mange [15] demonstrated no effect of inbreeding on fertility, measured by completed family size and birth rate. Only 2.3% of all Hutterite couples are childless (compared to an estimated 10% in the general population). Of all couples, 60% have their first child within the first year of marriage [19]. Spontaneous abortion rates have been estimated [19], but these estimates probably are spuriously low because Hutterites do not use pregnancy tests routinely and often reside in rural locales some distance from medical facilities. Consequently, many Hutterites probably would not distinguish between heavy menstruation and early fetal losses. Fetal losses recognized by Hutterites are thus likely to be those occurring only later in pregnancy. However, early fetal losses should increase intervals between births. Therefore, in this study, birth intervals were used to assess reproductive differences among Hutterite couples.

Sampling Procedures

Field trips were made to eight Hutterite colonies in 1976 and 1977 during our investigation of genetic factors in cancer [11–13]. Colonies were chosen on the basis of geographic location and relationship to ancestral colonies; none were chosen on the basis of information concerning reproductive histories or HLA types. Thus, no known sampling biases would be anticipated to affect interpretation of our results. During these field trips, medical and reproductive histories initially gathered by Steinberg et al. in the 1950s and 1960s [14] were updated. In five colonies, blood samples were obtained from all colony members who were older than age 5 and present during our visit. HLA-A and HLA-B were determined for 221 Hutterites. Approximately 120 antisera were used, defining most of the then (1977) known antigens. Subtypes of B12, Bw15, B17, Bw21, Bw22, and B40 could not be identified with confidence, thus analysis was performed only with the supertypic antigen. Typing was performed by a three-stage cytotoxicity technique [20].

HLA types were determined for both members of 50 of the 76 married couples in these colonies. Samples were not obtained from some husbands because they were either working in the fields or away from the colony on the day of our visit. Four of the 50 couples were eliminated from analysis for the following reasons: (1) one was excluded because the wife had been married previously, (2) one was excluded because the woman had a uterine lesion (probably leiomyomata) that when removed was followed by two normal pregnancies, and (3) two were excluded because they had been married less than 1 year.

There remained 46 couples who had been married more than 1 year. None were childless, and 11 had completed their families (i.e., wife was 45 years or older). These couples were subdivided on the basis of whether they shared zero (no. = 21), one (no. = 15), or more than one (no. = 10) HLA-A or HLA-B antigens. In the latter group, seven of the 10 couples shared haplotypes, two did not, and the haplotypes of one couple were not known.

Statistical Methods

Before comparing reproductive histories among couples sharing zero, one, or more than one HLA-A or HLA-B antigen, the three groups were compared for mother's age at marriage. A nonparametric test of comparison (Kruskal-Wallis one-way analysis of variance) was used because of our small sample size and because the distribution of age at marriage is slightly skewed.

Reproductive performance was assessed by calculating the intervals between term births (including the interval from marriage to first birth) and by measuring the intervals from marriage to each birth. Reliable information on the time when reproductive potential ceased in individual women was not available. Therefore, all couples were censored after their last term birth. These parameters were compared as described below.

(1) The distribution of birth intervals for each parity was compared using a nonparametric survival analysis [21]. This procedure compares the distribution of the probability that an event (i.e., births) will occur within a given time. It is particularly useful for analysis

TABLE 1
DISTRIBUTION OF PARITY AMONG 46 HUTTERITE COUPLES

SHARED HLA ANTIGENS	PARITY OF LAST BIRTH													TOTAL
	1	2	3	4	5	6	7	8	9	10	11	12	13	
0	1	0	1	3	5	0	4	1	1	0	2	1	2	21
1	1	1	0	1	1	2	1	4	1	0	2	0	1	15
> 1	0	0	0	0	0	1	2	0	2	2	3	0	0	9
Total.....	2	1	1	4	6	3	7	5	4	2	7	1	3	46

of censored data, for example, for incomplete families. Intervals were compared among the groups for the first through the eleventh parity; no couples with more than one antigen had more than 11 children (table 1). The log rank statistic was used for significance testing [21]. We chose to report medians rather than means as a summary statistic because medians tend to be more representative of highly skewed distributions, as is the case here.

(2) Median intervals from marriage to each birth were compared among the three groups. Survival analysis [21] was used as for the analysis of the distribution of birth intervals.

(3) Intervals from marriage to last birth for each couple (no. = 46) were compared using analysis of covariance, stratifying by zero, one, or more than one shared antigens and using parity as a covariate. Because the normal probability plot of the residuals showed no reason to seriously question the normality assumptions, variable transformations were not used. However, there was some evidence of heterogeneity of variance in other residual plots so the *P*-value must be treated as an approximation.

All analyses were performed on a CDC Cyber 170/730 computer at Northwestern University using the SPSS and BMDP statistical packages [22, 23].

RESULTS

Couples that shared zero, one, or more than one HLA-A or HLA-B antigen did not differ significantly with respect to age at marriage ($\chi^2 = .862, P = .650$). Survival analysis of birth intervals by parity revealed no significant differences among the three groups (*P* > .50 for all 11 parity groups) despite the observation that the median birth intervals were larger among couples sharing more than one antigen than among couples that share zero to one antigen in eight of 11 intervals examined (table 2). This reflects, at least in part, the small size of our sample.

Median intervals from marriage to first, fifth, and tenth births are presented in table 3. These intervals are consistently larger among couples sharing more than one antigen than among couples sharing zero or only one antigen. In addition, differences between the groups become larger with increasing parity even though the slopes of the regression lines were not statistically significantly different among the groups (*F* = .3004, *P* = .742). The slopes were essentially identical among couples that share zero or one antigen and were slightly larger among couples that share more than one antigen (figs. 1-4). The mean interval from marriage to last birth (adjusted for parity) for each couple was also different among the three groups (analysis of covariance based on a single slope: *F* = 2.762, *P* = .075), being greatest among couples with more than one shared antigen.

TABLE 2
 MEDIAN BIRTH INTERVALS (MONTHS) BY PARITY FOR COUPLES SHARING ZERO,
 ONE, OR MORE THAN ONE HLA-A OR HLA-B ANTIGEN

PARITY	SHARED HLA ANTIGENS		
	0	1	> 1
1	46.86(21)	43.86(15)	52.14(10)
2	69.86(20)	75.00(14)	85.71(10)
3	92.43(20)	75.00(13)	102.29(10)
4	74.71(19)	81.14(13)	72.00(10)
5	73.14(16)	81.29(12)	90.86(10)
6	66.00(11)	90.29(12)	79.86(9)
7	77.29(11)	101.43(9)	99.57(9)
8	83.29(7)	81.29(8)	87.57(7)
9	79.57(6)	78.43(4)	87.86(7)
10	92.14(5)	75.14(3)	157.14(5)
11	60.14(5)	79.57(3)	124.74(3)

NOTE: Nos. in parentheses are the no. cases.

Inbreeding coefficients were available for 31 of the 46 couples. As expected, there is some confounding of inbreeding levels (F) with degree of HLA sharing (means: $\bar{F}_0 = .0237$, $\bar{F}_1 = .0177$, $\bar{F}_{>1} = .0305$; medians: .0176, .0107, .0332), although there was no significant difference among the distributions of F in the three groups when classified as below or above the population mean ($\chi^2 = 3.7$, $.2 > P > .1$). Furthermore, previous work by Mange [15] revealed no effect of inbreeding levels on fertility in Hutterites. Nonetheless, the interaction of HLA sharing with inbreeding will be examined in more detail when larger samples are available.

Possible confounding effects of ABO and Rh incompatibility were examined among the 43 couples for whom data were available. Only three couples were Rh incompatible (wife Rh⁻, husband Rh⁺); thus, we did not compare reproductive performance between Rh-compatible and -incompatible couples. However, none of the three Rh-incompatible couples showed deleterious effects on reproductive outcome (i.e., late fetal losses or stillbirths). Fourteen couples were ABO incompatible (i.e., husband had an antigen that the wife lacked). In the 14 incompatible matings, the wife was blood group O and the husband A. No differences

TABLE 3
 MEDIAN INTERVAL (YRS) FROM MARRIAGE TO EACH BIRTH AMONG COUPLES
 SHARING ZERO, ONE, OR MORE THAN ONE HLA-A OR HLA-B ANTIGENS

PARITY	SHARED HLA ANTIGENS		
	0	1	> 1
190(21)	.86(15)	1.00(10)
5	6.98(16)	7.17(12)	7.42(10)
10	13.73(5)	14.52(3)	19.00(5)

NOTE: Nos. in parentheses are the no. cases.

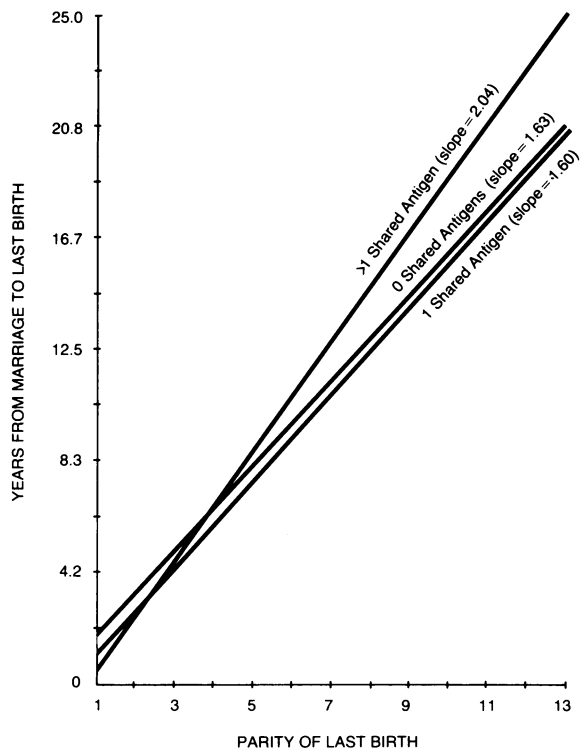


FIG. 1.—Relationship between years from marriage to last birth and parity of last birth among couples sharing zero (no. = 21), one (no. = 15), and more than one (no. = 10) HLA-A or HLA-B antigens.

were found between ABO-compatible (no. = 29) and ABO-incompatible (no. = 14) couples, judged by the length of interval from marriage to last birth, using parity as a covariate (comparison of means: $F = 1.78$, $P = .190$; comparison of slopes: $F = 0.014$, $P = .908$). However, the small size of our sample precluded examining interactive effects between the *HLA* and *ABO* loci; therefore, this cannot be excluded as a possibility.

DISCUSSION

Our data are consistent with the hypothesis that shared HLA antigens affect reproductive outcome deleteriously, but introduce a strong note of caution about their clinical implications. Analyses of birth intervals showed small, yet consistent, differences among couples that share zero, one, or more than one HLA-A or HLA-B antigen. Couples sharing more than one antigen have not only longer birth intervals but fewer offspring per year at risk than do couples sharing zero or only one antigen. In our sample, couples with more than one shared antigen required 10% longer to produce their first offspring and 20% longer to produce 10 offspring, compared to couples with zero or one shared antigen. Figure 1 further shows the relationship among the three groups between the length of

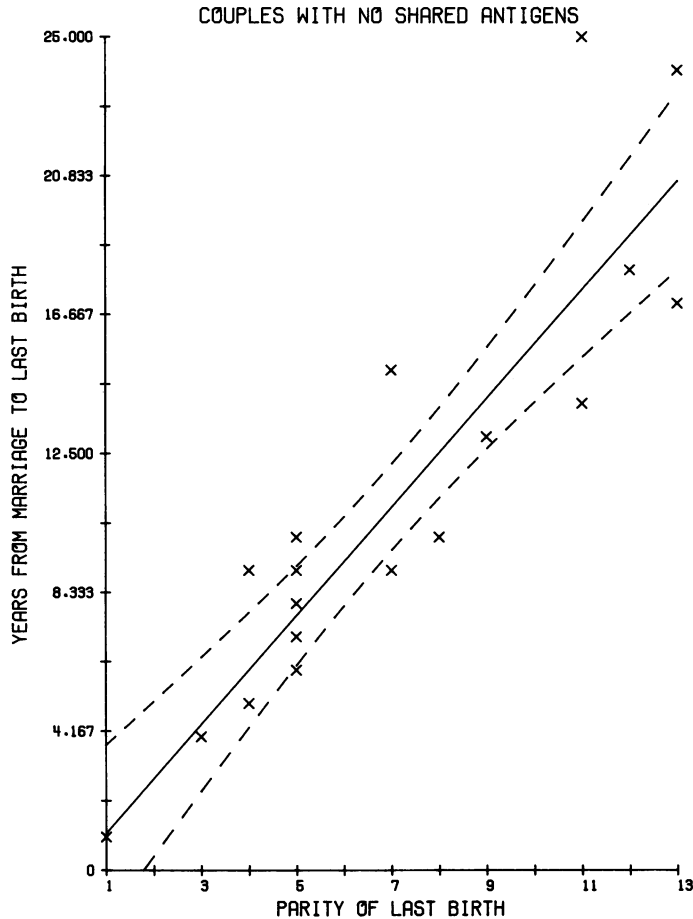


FIG. 2.—Relationship between years from marriage to last birth and parity of last birth among couples sharing zero (no. = 21) HLA-A or HLA-B antigens. Dotted lines represent the 95% confidence interval.

interval from marriage to last birth (Y) and parity of last birth (X). Although the difference in slopes is not statistically significant, it does lead to larger differences between the lines with increasing parity, suggesting that any deleterious effect of shared antigens may become greater in later pregnancies; that is, maternal sensitization to paternally derived antigens may occur in early pregnancies followed by a greater selective advantage to histoincompatible fetuses in later pregnancies. This is consistent with observations of excesses of mice heterozygous at the *H-2* loci in third and fourth litters but not in first and second litters [24] and suggest that deviations from expected segregation ratios for HLA antigens may be detectable only in human populations with large family sizes.

Reduced fertility due to increased early fetal loss rates in couples that share antigens could have important evolutionary implications. MacCluer [25, 26] used computer simulation models to investigate the impact of early fetal losses on

Darwinian fitness. MacCluer found that a 30% increase in the fetal loss rate resulted in a 10% increase in the length of the interval from marriage to first birth, an increase comparable to that observed in our sample (table 3). This 30% increase in fetal loss rates resulted in a 14% decrease in overall fitness in using this model. If histoincompatible fetuses are at an advantage during gestation, selection at these loci may be frequency-dependent. This selection could perpetuate variability and maintain polymorphism at histocompatibility loci by favoring new mutations and rare alleles.

These evolutionary implications notwithstanding, what are the immediate clinical implications, especially for couples with repetitive spontaneous abortions of unknown etiology? It has been proposed that women who have experienced early fetal losses and who share HLA antigens with their husbands be treated by im-

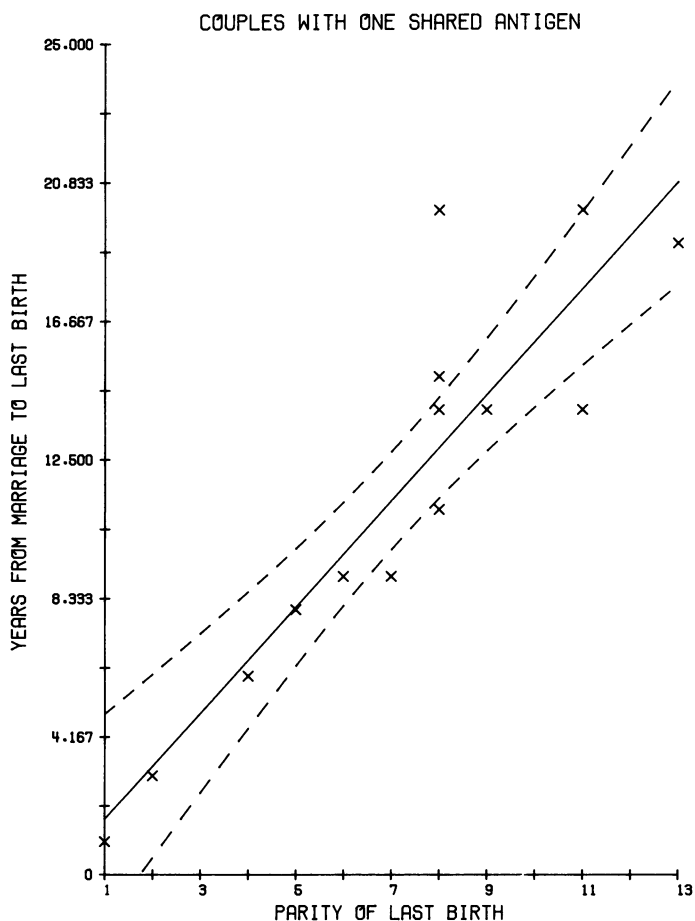


FIG. 3.—Relationship between years from marriage to last birth and parity of last birth among couples sharing one (no. = 15) HLA-A or HLA-B antigen. *Dotted lines* represent the 95% confidence interval.

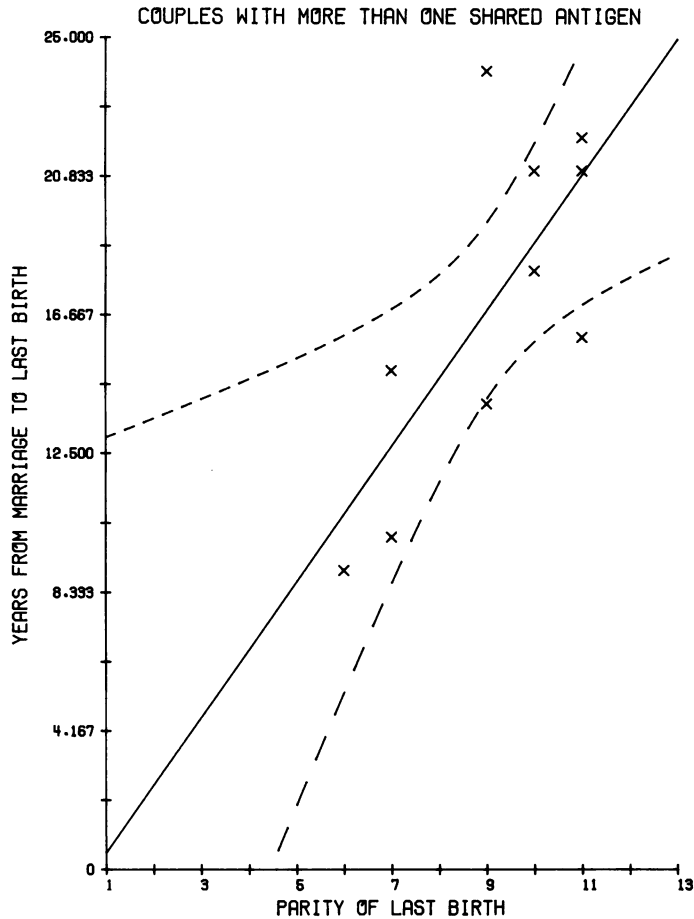


FIG. 4.—Relationship between years from marriage to last birth and parity of last birth among couples sharing more than one (no. = 10) HLA-A or HLA-B antigen. *Dotted lines* represent the 95% confidence interval.

munizations or repeated leukocyte transfusions prior to and during pregnancy [10, 27]. Indeed, this approach may be successful; however, recommendations for this treatment are based on retrospective studies of couples experiencing repetitive spontaneous abortions, often studies with inadequate controls. By contrast, our results demonstrate that sharing HLA antigens does not preclude normal pregnancy, although sharing antigens is not necessarily innocuous. Our study indicates that caution should be exercised before concluding that shared HLA antigens are solely responsible for repeated fetal losses, even after no other explanation [28] is evident.

ACKNOWLEDGMENTS

We thank the Hutterites for their courtesy and cooperation, Dr. Arthur G. Steinberg for his foresight and pioneering spirit that made this work possible, and Dr. Claire Gordon for providing us with unpublished data on the Dariusleut.

REFERENCES

1. GILL TJ, REPETTI CF: Immunologic and genetic factors influencing reproduction. *Am J Pathol* 95:464–570, 1979
2. BEER AE, BILLINGHAM RE: *The Immunology of Mammalian Reproduction*. Englewood, N. J., Prentice-Hall, 1976
3. BEER AE, BILLINGHAM RE: Mechanisms of non-rejection of foeto-placental allografts. *Folia Biol (Praha)* 26:225–243, 1980
4. BILLINGTON WD: Influence of immunologic dissimilarity of mother and foetus on size of placenta in mice. *Nature* 202:317–318, 1964
5. JAMES DA: Effects of antigenic dissimilarity between mother and foetus on placental size in mice. *Nature* 205:613–614, 1965
6. KIRBY DRS: The egg and immunology. *Proc R Soc Med* 63:59–61, 1970
7. KOMLOS L, ZAMIR R, JOSHUA A, HALBRECHT I: Common HLA antigens in couples with repeated abortions. *Clin Immunol Immunopathol* 7:330–335, 1977
8. SCHACTER B, GYRES M, MUIR A, TASIN M: HLA-A, B compatibility in parents of offspring with neural-tube defects on couples experiencing involuntary fetal wastage. *Lancet* April 14, 1979
9. BEER AE, GAGNON M, QUEBBEMAN JF: Immunologically induced reproduction disorders, in *Endocrinology of Human Infertility, New Aspects*, edited by CROSIGNANI PG, RUBIN BL, London, Academic Press, 1981, pp 419–439
10. BEER AE, QUEBBEMAN JF, AYERS JWT, HAINES RF: Major histocompatibility complex antigens, maternal and paternal immune responses, and chronic habitual abortions in humans. *Am J Obstet Gynecol* 141:987–997, 1981
11. MARTIN AO, DUNN JK, SIMPSON JL, ET AL.: Cancer mortality in a human isolate. *J Natl Cancer Inst* 5:235–255, 1980
12. MARTIN AO, DUNN JK, SMALLEY B: Use of a genealogically linked data base in the analysis of cancer in a human isolate, in *Banbury Report 4: Cancer Incidence in Defined Populations*, edited by CAIRNS J, LYON JL, SKOLNICK M, Cold Spring Harbor, N.Y., Cold Spring Harbor Laboratory, 1980, pp 235–257
13. MARTIN AO, DUNN JK, SIMPSON JL, ET AL.: Genetics of neoplasia in a human isolate, in *Genetic and Environmental Factors in Experimental and Human Cancer*, edited by GELBOIN HV, MACMAHON B, MATSUSHIMA T, SUGIMURA T, TAKAYAMA S, TAKEBE H, Tokyo, Japan Scientific Society Press, 1980, pp 291–302
14. STEINBERG AG, BLEIBTREU HK, KURCZYNSKI TW, MARTIN AO, KURCZYNSKI EM: Genetic studies in an inbred human isolate, in *Proceedings of the Third International Congress of Human Genetics*, edited by CROW JF, NEEL JV, Baltimore, Johns Hopkins Univ. Press, 1967, pp 267–290
15. MANGE AP: Growth and inbreeding of a human isolate. *Hum Biol* 36:104–133, 1964
16. BLEIBTREU HK: Marriage and residence patterns in a genetic isolate. Ph.D thesis, Cambridge, Harvard Univ., 1964
17. HOSTETLER JA: *Hutterite Society*. Baltimore, Johns Hopkins Univ. Press, 1974
18. OLSEN CL: The demography of new colony formation in a human isolate: analysis and history. Ph.D. thesis, Ann Arbor, Univ. of Michigan, 1976
19. SHEPS MC: An analysis of reproductive patterns in an American isolate. *Popul Stud* 19:65–80, 1965
20. AMOS DB, POOL P, GRIER J: HLA-A, HLA-B, HLA-C, and HLA-DR, in *Manual of Clinical Immunology*, edited by ROSE NR, FRIEDMAN H, Washington, D.C., American Society for Microbiology, 1980, pp 978–986
21. PETO R, PIKE MC, ARMITAGE P, ET AL.: Design and analysis of randomized clinical trials requiring prolonged observation of each patient. II. Analysis and examples. *Br J Cancer* 35:1–39, 1977
22. DIXON WJ, ED: *BMDP Statistical Software*. Los Angeles, Univ. of California Press, 1981
23. HULL CH, NIE NH: *SPSS*. New York, McGraw-Hill, 1979

24. HULL P: Maternal-fetal incompatibility associated with the H-3 locus in the mouse. *Heredity* 24:203-209, 1969
25. MACCLUER JW: Fertility and mortality effects on Darwinian fitness in man. *Hum Biol* 51:391-410, 1979
26. MACCLUER JW: On the probability of demonstrating differential fertility in genetic studies. *Ann Hum Gen* 42:59-75, 1978
27. TAYLOR C, FAULK WP: Prevention of recurrent abortion with leukocyte transfusion. *Lancet* July 11, 1981
28. SIMPSON JL: Repeated suboptimal pregnancy outcome. *Birth Defects: Orig Art Ser* 17(1):113-142, 1981