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REPRODUCTIVE OUTCOMES IN THE WOMEN OF THE RED RIVER VALLEY OF THE NORTH. I. THE SPOUSES OF PESTICIDE APPLICATORS: PREGNANCY LOSS, AGE AT MENARCHE, AND EXPOSURES TO PESTICIDES

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In the current study, there was a modest but significant increase in risk (1.6- to 2-fold) for miscarriages and/or fetal loss occurring throughout the year in the spouses of applicators who use fungicides. There is a surprisingly significant deficit in the number of male children born to the spouses of fungicide applicators. First-trimester miscarriages occur most frequently in the spring, during the time when herbicides are applied. Use of sulfonylurea (odds ratio OR = 2.1), imidizolinone (OR = 2.6) containing herbicides, and the herbicide combination Cheyenne (OR = 2.9) by male applicators was statistically associated with increased miscarriage risk in the spring. Limited survey data from women who are the spouses of applicators did not show major alterations of long-term endocrinologic status (menarche, menopause, endometriosis). With regard to personal pesticide exposures, only women who engaged in pesticide application where there is direct exposure to these products are at demonstrable risk (OR = 1.8) for miscarriage. It was hypothesized that the overall reproductive toxicity observed in this population is, for the greater part, a male-mediated event. Clarification of exposure events leading to reproductive toxicity through direct measurements of exposure in both men and women is needed to resolve this issue.

In humans, fetal loss through miscarriage, prematurity, or still birth is frequent. Human population studies indicate that the reported frequency of these untoward reproductive events are subject to a high degree of variability (Gerhard et al., 1998; Lewis 1997; Rempen, 1993; Goldhaber & Fireman, 1991; Stellman et al., 1988). In this connection, use of laboratory-based detection methods, awareness of outcome, and self-reporting bias are all issues of concern and could account for some differences noted (Wilcox et al., 1988; Gold & Tomich, 1994; Scialli et al., 1997). In studies of occupation, local urban or rural environmental pollution, expected bias in self-reported health status, and exposure assessment add further complexity (Gold & Tomich, 1994; Scialli et al., 1997; Goldman 1997; Kavlock & Setzer, 1996; Vassilev et al., 2001). Despite these noted difficulties, the relatively high frequency of occurrence of these adverse reproductive

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health outcomes provides an opportunity for more detailed examination of different occupational exposure scenarios within a more general exposed population. Further, internal comparisons among broad based exposure groups within the same population would be expected to minimize recall bias.

Earlier studies by Savitz et al. (1997) demonstrated the utility of this approach in regard to paternal use of different classes of pesticides and the occurrence of spontaneous abortions. These studies suggested that paternal exposure and use of dithiocarbamate pesticides and combinations of other pesticides led to significant increases in the spontaneous abortion rate among farm women. In a somewhat similar vein, laboratory-based population studies by our group demonstrated that pesticide applicators using only herbicides showed the lowest level of chromosomal damage compared to applicators who applied insecticides and fumigants (Garry et al., 1996a). Applicators who applied fumigants and/or insecticides showed significant increases in chromosome aberrations, including chromosome rearrangements, compared to unexposed controls or those who only applied herbicides (Garry et al., 1996a). In vitro cellular and molecular studies of pesticides in common use in the Red River Valley suggested that dithiocarbamate fungicides (maneb, mancozeb) and the fungicide triphenyltin might induce apoptosis in early development (Lin & Garry, 2000). In these same in vitro studies (Lin & Garry, 2000), adjuvants used in conjunction with herbicides, such as 2,4-D, were found to have weak pseudo-estrogenic effects. Taking these findings together, it was hypothesized that the female spouses of applicators who applied fungicides and other products might be at higher risk for fetal loss. Further, alterations of the endocrine status of women exposed to herbicides might be reflected in the frequency of menstrual-cycle irregularities and differences in the age at menarche. The current report examines these hypotheses as part of our overall effort to evaluate in detail previously reported adverse reproductive effects and cancer in the farming community of the Red River Valley (Garry et al., 1996b; Schreinemachers et al. 1999).

MATERIALS AND METHODS

Population and Population Access

In Minnesota, licensing for application of pesticides either commercially or for application to one's own farmland requires periodic recertification by completion of a program of education and examination. Through the Minnesota Department of Agriculture and the Minnesota Extension Service, an updated list of licensees was provided covering the time frame from 1991 through 1996.

Survey Strategy

In the five-county Red River Valley study area, 3000 residents were licensed during the time frame cited. One thousand five hundred persons were randomly selected for our umbrella study of general health and pesticide use. The locations of 1340 licensees were confirmed through phone and postcard follow-up. One thousand and seventy applicators (98% male) volunteered to participate by phone interview for the general health and pesticide use study. Of this group, 851 were married or had a marriagelike relationship. Eight hundred and two spouses (women) participated in the general health and pesticide use survey.

Reproductive Health Assessment

During the phone interview, applicators and/or their spouses were also invited to participate and return a detailed written reproductive health and pesticide use assessment questionnaire. Informants for the reproductive study were 228 male spouse only (pesticide applicator), 90 female spouse only, and 377 couples. Thus, at least one member of 695 families responded and gave detailed information regarding reproductive health and pesticide use. Of the 695 families, self-reports from 133 informant families indicated that they did not have children. Of this group, 54 stated they did not want to have children, 15 stated difficulty having children, and 64 gave no detailed statement in regard to childbearing status. In toto, this stepped approach to detailed reproductive health survey met University of Minnesota Institutional Review Board (IRB) approval and concerns regarding privacy and data reporting.

Eligibility Criteria

All self-reported pregnancies fathered by a pesticide applicator ($n = 540$) were considered in this study. Families who had twin or other multiple pregnancies and pregnancies associated with other specific risk factors depicted below were excluded from detailed study ($n = 18$). Thus, pregnancies fathered by 522 applicators were considered in our final data analysis.

Pregnancy and Pregnancy Outcomes

At the time of the survey, all pregnancies fathered by each applicator throughout his reproductive life-span, including current pregnancy, were included in our assessments. Live births, children with birth defects, and all fetal losses were defined by written survey. Those participants indicating a fetal loss were again contacted by mail and/or phone to provide details regarding the pregnancy loss. First- and second-trimester miscarriages (28 wk gestation or less), season in which they occurred, and year were ascertained. Stillbirths were defined as deaths beyond 28 wk gestation, and deaths due to premature birth (28 wk to 37 wk gestation) were enumerated (Sever et al., 1997). Ectopic and tubal pregnancies were identified. Induced abortions were not specifically identified and were included as "other."

Pregnancy Losses Excluded From Detailed Statistical Analysis

Ectopic or tubal pregnancies, induced abortions, and families with twin or other multiple pregnancies were excluded from detailed statistical analyses of fetal loss and miscarriages.

Subjects with recurrent miscarriages specifically related to anatomically defined pathologic entities, such as pelvic inflammatory disease or incompetent cervix, were excluded from detailed statistical analysis.

Miscarriages arising in association with treatment with fertility drugs were also excluded.

Pesticide Use

Each certified pesticide applicator (98% male) was initially interviewed by phone regarding current and past pesticide use with specific attention to product name and the number of days per year applied. Approximately 6 mo later, where possible, the subject was reinterviewed by written questionnaire to document common pesticide use by pesticide class, acreage treated, type of crop, and use of personal protective gear. Overlap between the two questionnaires was intentional to validate use of pesticides by class (herbicides, insecticides, fumigants, and fungicides). Similarly, the spouse of the applicator (98% female) was interviewed by phone and again by written questionnaire. Involvement in the processes associated with pesticide use historically and currently was assessed. Personal use of pesticides by spouses was also ascertained. Where possible, specific pesticide exposure and/or use was identified.

Statistics

Adjusted odds ratios were calculated using logistic regression. Indicator variables were coded for the following categories: five pesticide use groups with herbicide only as the referent group, maternal age (years) at the time of pregnancy (ages 15–24, ages 25–34 as referent, >35, no age given), smoking status (current, ex-smoker, nonsmoker as referent), and drinking alcoholic beverages (rarely/never as referent). The chi-square test for significance was used to assess sex ratio and seasonal differences between pesticide use groups. Standard *t*-tests were used to examine differences in reproductive years. Age at menarche and decade of birth were evaluated using linear regression and Pearson's correlation. Chronic diseases such as diabetes, pharmacologically treated hypertension and arthritis, and occupations other than agriculture were considered separately. Specific medication use during pregnancy and dietary information were not considered in our survey. Residence in a rural site (towns less than 3000 population) or on a farm during childhood (less than age 18 yr) were considered factors in some of these statistical analyses.

RESULTS

Male Pesticide Use, Fetal Loss, and Miscarriages

Table 1 lists the frequency of fetal loss according to the classes of pesticides applied by the male applicator. In this study group, 540 farm families with children, where the applicator was the biologic father or whose

TABLE 1. Pregnancies and Pregnancy Losses Among the Spouses of Pesticide Applicators

Pesticide use group	Number of applicators	Total pregnancies (*n*)	Pregnancy losses[b] (*n*)	Losses/ pregnancy (%)	Odds ratio	95% CI
Herbicide only (referent)	110	338	24	7.10	1	
Herbicide/insecticide	72	226	20	8.85	1.27	0.68–2.36
Herbicide/insecticide/fungicide	180	565	63	11.15	1.64	1.01–2.67
Herbicide/insecticide/fumigant	36	110	9	8.18	1.17	0.53–2.59
All four pesticide classes	66	213	17	7.98	1.14	0.59–2.17
Other[a]	58	150	11	7.33	1.04	0.49–2.17
Total	52	1602	144	8.99		

Note. Listed are the numbers of pregnancies and fetal losses (miscarriages, stillbirths and deaths in premature infants) listed according to male applicator pesticide use practice by pesticide class. Odds ratios (OR) and confidence limits for each pesticide use group are based on comparison to applicators who only used herbicides (referent group). The results recorded are from singleton pregnancies. As a group, the spouses of applicators who apply herbicides, insecticides, and fungicides have significantly more miscarriages ($p \leq .05$).

[a]Other includes pesticide combinations other than those listed (e.g., herbicide/fumigant, herbicide/fungicide).

[b]Pregnancy losses includes miscarriages, stillbirths, and premature death; excludes multiple pregnancies (e.g., twins), excludes nonapplicator paternity.

spouse had been pregnant in the relationship, participated and gave detailed pesticide use data. On average, these families had 2.8 children per couple. Divided according to application practice (Table 1), these families had approximately 3 pregnancies per couple except for those classified as "other" by pesticide use (approximately 2.6 pregnancies per couple). One hundred and thirty-three families did not report pregnancies or births. Of those couples who had children or who had been pregnant, one in four women had a miscarriage. In the more detailed analysis show in Table 1, miscarriages associated with specific pregnancy risk factors were excluded, leaving 522 applicator families eligible for study. Spouses of pesticide applicators who used herbicides, insecticides, and fungicides had more miscarriages than any other pesticide application group. Logistic regression examination of potential confounders including smoking and drinking patterns among the female spouses did not significantly affect these results (unadjusted OR = 1.64, CI 1.00–2.67; adjusted OR = 1.68, CI 1.02–2.70). Maternal age over 35 yr was considered an independent risk factor in these analyses (adjusted OR for age over 35 = 1.98, CI 1.04–3.77). Paternal smoking, drinking, and paternal age, similarly, were not significant factors in these results.

Characterization of Pesticide Use Groups

In Minnesota (Garry et al., 1996b), herbicides are routinely applied to crops in spring, insecticides in summer, and fungicides as needed in summer and fall. The mean acreage treated with pesticides per year is highest among applicators who use herbicides, insecticides, and fungicides (Table 2). The total treated acreage in each exposure group reflects applications of different classes of pesticides sequentially during spring, summer, and fall to the same land. In this connection, pesticide applications may be delivered by ground or aerial methods. Sixteen percent or less of the applicators in the herbicide-only group employed aerial application: 27% in the combined herbicide/insecticide use group and 65% of applicators employed aerial applications in the herbicide/insecticide/fungicide use group. Only 74% (384/522) of applicators gave these detailed responses regarding acreage and pesticide use. Interestingly, 85% of the herbicide/insecticide/fungicide group provided detailed information, while only 64% of the herbicide-only group were responsive. The majority (65%) of the nonrespondents in the herbicide only group were engaged in noncrop work or were not currently applying pesticides. Complementary but less detailed data from our general health and pesticide use survey conducted by phone was consistent with these pesticide use patterns. In that data set, 28% of the participants applied fungicides and gave information regarding pesticide use (804 of 1054 participants or 76% of all participants applied fungicides themselves). Twenty-two percent of the group who applied fungicides themselves performed aerial applications. Forty-eight percent of pesticide applicators surveyed also had fungicides applied to their farm lands by others. Eighty-one percent of these applications were delivered to the land by the aerial route (data not shown).

Specific Fungicide Use

Regarding specific fungicide use (Table 3), the unadjusted odds ratio for specific use of organotin (OR = 1.55; CI 1.01–2.37) and/or use of EBDC (ethylene bisdithiocarbamate) fungicides (OR = 1.77; CI 1.11–2.83) such as mancozeb demonstrates a significantly increased frequency of fetal loss compared to nonuse of fungicides by applicators. The magnitude of the increase and/or difference in the values of the odds ratio for these two fungicides do not allow for further specification of risk. Specific use of chlorophenoxy herbicides and no fungicides yielded a 7% fetal loss, not significantly

TABLE 2. Acreage Treated with Pesticides and Use of Aerial Application

Pesticide class	Number of respondents	Mean of total acreage treated	Percent use aerial application
Herbicide only	70	1290	16
Herbicide/insecticide	55	1296	27
Herbicide/insecticide/fungicide	155	3142	65
Herbicide/insecticide/fumigant	25	1348	37
All four classes	51	2352	68
Other	58	1773	39

Note. Compared according to pesticide class use are the average acreages to which pesticides were applied. Aerial applications occurred most frequently when fungicides were applied.

TABLE 3. Pregnancy Loss and Specific Fungicide Use

Fungicide used	Users (*n*)	Pregnancies (*n*)	Total losses (*n*)	Losses/ pregnancy (%)	Odds ratio	95% CI
Organotin	105	335	39	11.64	1.55	1.01–2.37
Triazole	52	154	18	11.69	1.56	0.89–2.72
EBDC[a]	72	222	29	13.06	1.77	1.11–2.83
Substituted aromatics[b]	15	38	3	7.89	1.01	0.30–3.39
Benzimidazoles	24	68	4	5.88	0.74	0.26–2.09
No fungicide use (referent)	243	741	58	7.83	1	

Note. Identified are the numbers of applicators who use each class of fungicide listed, pregnancies among their spouses, and numbers of fetal loses. Spouses of applicators who use or apply organotin (e.g., triphenyltin) or EDBC-containing fungicides (maneb or mancozeb) are at increased risk for miscarriage compared to the referent group (no use of fungicides). The spouses of applicators who apply thiazole-containing fungicides show a suggestive increase in miscarriage risk compatible with chemical conversion of thiazoles to EDBCs. Application of substituted aromatic fungicides such as hexachlorobenzene or use of benzimidazoles such as benomyl is infrequent.

[a]EDBC, ethylene bisdithiocarbamate-containing fungicides (maneb and mancozeb).

[b]Substituted aromatics, such as Bravo, Daconil, Terranil.

different from the referent group (7.8%). More detailed analyses of herbicide use according to season yield different results.

Maternal Pesticide Exposure

Maternal use and exposure to pesticides was also examined. Of the 467 women who had returned the reproductive health survey, 379 had been pregnant or had children. Two hundred and sixty-nine women noted aerial applications to the resident farm or to neighboring farms. One hundred and twenty-six women carried food to pesticide-treated fields within 48 h of application, and 315 out of 379 women participants washed pesticide-treated clothing. None of these activities significantly affected the frequency of fetal loss. However, personal use of pesticides, including, mixing, loading and pesticide applications by the female spouse, was a significant risk factor for fetal loss (fetal loss/pregnancy OR = 1.81; CI 1.04–3.12). Only 36 of 379 women respondents applied or mixed pesticides. Thirty percent had a fetal loss compared to 21% of those women who did not mix or apply pesticides. The number of fetal losses per pregnancy is significantly elevated in those who did mix or apply pesticides (15% vs. 8.9%; data not shown).

Miscarriage and Season

Figure 1 compares, by pesticide class use, the number of first trimester miscarriages per first-trimester pregnancy according to season. The frequency of first-trimester miscarriages regardless of pesticides applied demonstrates

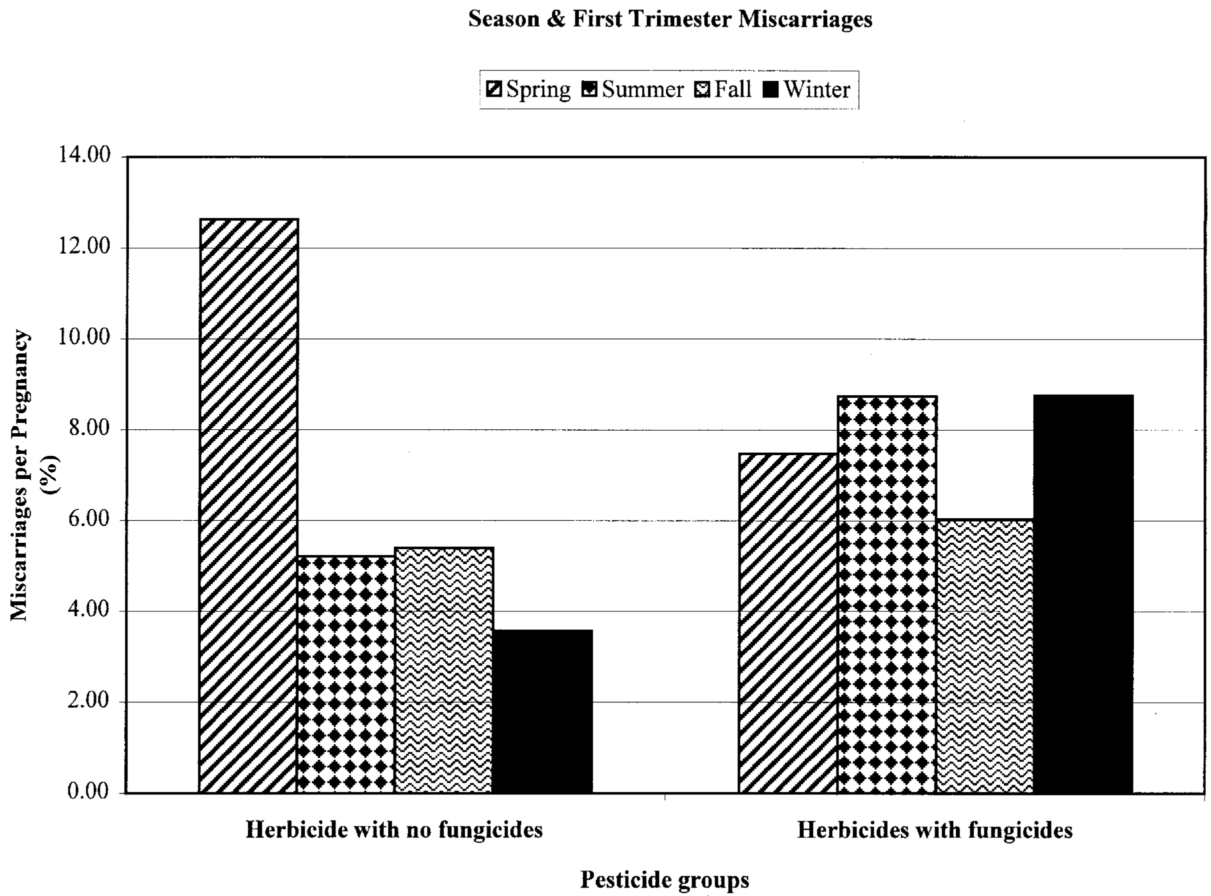


FIGURE 1. The highest frequency (p = .007) of first-trimester miscarriages occurring in the spring was observed among the spouses of applicators who apply herbicides but not fungicides, compared to all other spouses. The frequency of all spring first-trimester miscarriages regardless of pesticide use is also significantly elevated in spring, compared to all other seasons (p = .034). The p values recorded are the results from Fisher's exact test.

that in the spring the number of first trimester miscarriages is significantly elevated ($p = .034$) compared to all other seasons. Within-group comparison (herbicide use but no fungicides) shows similar results ($p = .007$) for spring. Further, spouses of applicators who apply fungicides and other pesticides show no such peak in the miscarriage frequency in spring. However, the frequency of first-trimester miscarriages is significantly higher in summer, fall, and winter combined for the spouses of applicators of fungicides ($p = .05$) in comparison to applicator families whose male spouse does not apply fungicides.

As listed in Table 4, comparison of six commonly used classes of herbicides or herbicide combinations, according to use or nonuse of these herbicide groups, shows that pregnancies of the spouses of applicators who applied one or more of the herbicide groups of sulfonylureas, imidizolinone, or mixture 9100 were at significantly increased risk for a miscarriage in spring.

The number of applicators who applied these herbicides and whose spouse did or did not have a miscarriage is also of interest. Spouses of 35 applicators had a total of 40 miscarriages in the spring. Chlorophenoxy herbicides were the most frequently used herbicide class accounting for 62.7% (156/249) and 71.4% (25/35) of applicator herbicide use in either group (without miscarriage and with miscarriage). The miscarriage rate expressed as miscarriages per pregnancy was the lowest (11.5%) in the chlorophenoxy

TABLE 4. Spring Miscarriages and Herbicide Use

Herbicides by chemical class used in spring	Applicators (total = 284) using herbicide	Pregnancies (total = 399) conceived in spring	Miscarriages ($n = 40$) in spring	Miscarriage/ pregnancy (%)	Miscarriage risk (odds ratio) comparison of herbicide used to all other pesticides	
					OR	95% CI
Chlorophenoxy	181	253	29	11.5	1.59	0.77–3.27
Sulfonylurea	77	110	17	15.5	2.11	1.09–4.09
Imidizolinone	30	40	8	20.0	2.56	1.11–5.87
Oxyphenoxy	75	105	13	12.4	1.40	0.69–2.82
Mixture 9101[a]	52	70	10	14.4	1.66	0.78–3.56
Mixture 9100[b]	35	52	11	21.2	2.94	1.40–6.16

Note. Commonly used herbicides are listed according to chemical class, along with herbicide combinations, applied by applicators whose spouse had a spring miscarriage. The number of first-trimester spring miscarriages and the number of male spouses (applicators) who used these products are shown. The risk of miscarriage was significantly higher among the spouses of applicators who used herbicide products containing sulfonylureas, imidizoline, and/or mixture 9100.

[a]Chlorophenoxy + bromophenol.

[b]Chlorophenoxy + sulfonylurea + benzothiodiazole.

use group. In contrast, 6/35 (17.1%) applicators whose spouse had a miscarriage and 24/249 (9.6%) whose spouse did not have a spring miscarriage used imidizolinone. Similarly, 7/35 (20.0%) applicators versus 28/249 (11.2%) who did not have a spring miscarriage used mixture 9100 and 13/35 (37.1%) applicators versus 64/249 (25.7%) used sulfonylurea. These data comparisons do not achieve 95% confidence interval for significance; however, they show a consistent pattern of effect in support of the comparisons made and reported in Table 4.

Live Births and Sex Ratio

Table 5 depicts the number of live births and the sex of children borne to pesticide applicator families by pesticide use group. Compared to all other pesticide use groups, the sex ratio of children borne to applicators applying fungicides, insecticides, and herbicides is significantly altered ($p = .02$) compared to the referent group (herbicide only). Approximately 21% fewer boys than girls were borne to families in this pesticide use group.

Age at Menarche, Reproductive Life Span, and Endometriosis

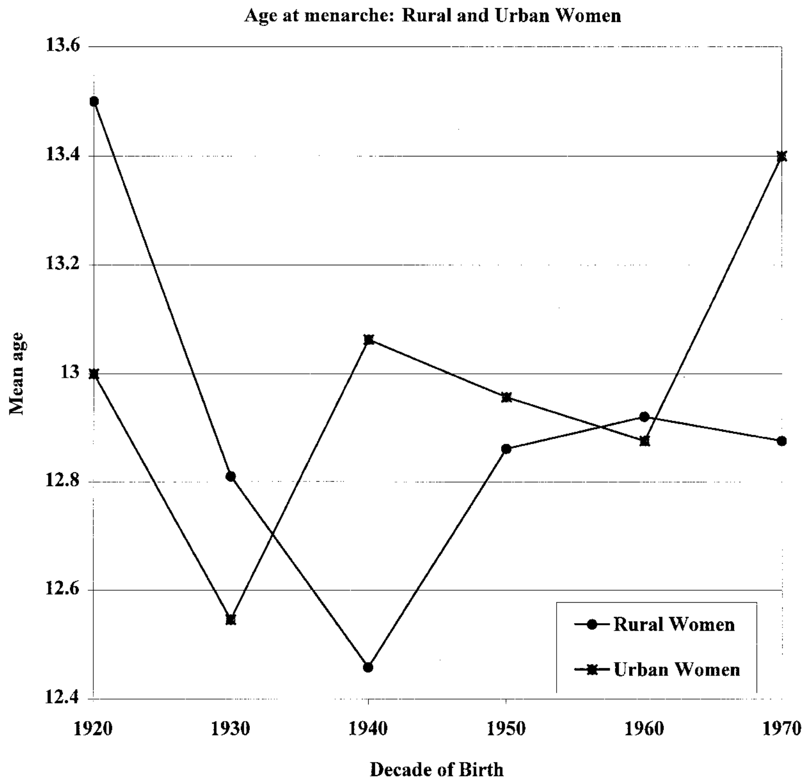
The age at menarche for rural women in this study seemed to vary according to decade of birth (Figure 2). Menarche in rural women borne in the decades from 1920 through 1949 shows a decrease from 13.5 ± 0.62 yr (1920–1929) to a minimum of 12.5 ± 0.81 yr (1940–1949). The downward trend (slope = –0.040) in age at menarche is significant (Pearson's correlation $p = .03$) for women who lived in a rural setting before age 18 yr. From 1949 onward there is an upward trend in age at menarche (slope = +0.016) but these data are not significant ($p = .11$). Regardless of decade, the mean

TABLE 5. Sex of Children and Pesticide Use

Applicator type	Total live births (*n*)	Male (*n*)	Female (*n*)	M/F ratio	*p* Value (≤)
Herbicide only (referent)	342	181	161	1.12	Referent
Herbicide/insecticide	209	110	99	1.11	.95
Herbicide/insecticide/fungicide	508	226	282	0.80	.02[a]
Herbicide/insecticide/fumigant	106	57	49	1.16	.88
All four classes	187	102	85	1.20	.72
Other	134	72	62	1.16	.98
Total	1486	748	738	1.014	

Note. The total number of pregnancies resulting in liveborn male and female children are listed according to male applicator pesticide use (herbicides, insecticides, fumigants, and fungicides). Pregnancies and children born to applicator families where the male spouse applies only herbicides (referent group) are compared to all other pesticide use groups. There are significantly fewer male children born to the spouse of men applying herbicides, insecticides, and fungicides.

[a]Chi-square test for significance.



		1920-29	1930-39	1940-49	1950-59	1960-69	1970's
Rural Women	N=	12	42	59	115	75	16
Urban Women	N=	2	12	32	46	40	5

FIGURE 2. The trend in age at menarche shows a consistent downward trend in rural women born between 1920 to 1949, with a reciprocal rise in the age at menarche for rural women born from 1959 to 1979. Exclusion of women who lived in nonfarm setting before age 18 yr enhances these cyclic differences in age at menarche.

in age at menarche appears more variable among women who lived in an urban environment until age 18 yr. These data are also relatively sparse and led to no detectable trend in the age at menarche.

The reproductive life span (menarche to natural menopause; Table 6) among women who lived on farms or rurally (36.5 yr) was somewhat shorter than for women currently living in an urban setting (38.9 yr). This 2.4-yr difference in reproductive life-span was significantly different (p = .02). However, comparison of the reproductive life span of women who lived on a farm during childhood to those who grew up in an urban setting showed a nonsignificant 1.1-yr difference. The frequencies of endometriosis and menstrual irregularity are not different between rural and nonrural women in either data set.

TABLE 6. Reproductive Life Span, Menstruation, and Endometriosis: Farm and Urban Women

Menopause[a]	*n*	Menarche age (yr)	Menopause age (yr)	Reproductive years[b]
Rural	62	12.8 ± 1.3	49.3 ± 5.3	36.5 ± 5.3
Urban	18	13.0 ± 1.4	51.8 ± 2.9	38.9 ± 3.1
Menstruation	***n***	**Regular menses**	**Irregular menses**	**Always irregular**
Rural	269	38.3%	61.7%	13.4%
Urban	107	30.0%	70.0%	16.8%
Endometriosis	***n***			
Rural	32	9.6%		
Urban	9	7.2%		
Total respondents	457			
Rural	332			
Urban	125			

Note. Shown are the average ages of menarche and "natural" menopause for women born and/or lived as a child in rural or nonrural settings. Women who live in a rural setting have a somewhat shorter reproductive lifespan (two-sided *t*-test for significance, $p = .02$). There are no survey-driven significant differences in the frequency of menstrual-cycle irregularities, nor is the frequency of endometriosis any different among rural and nonrural women of the Red River Valley.

[a]Natural menopause.

[b]Using *t*-test for unequal variances, $p \leq .02$.

DISCUSSION

Miscarriage Rate and Sex Ratio

As indicated earlier, the reported findings by Savitz et al. (1997) are remarkably similar in a number of respects to the data we now report (Tables 1–3). In that study, specific use of dithiocarbmate fungicides was associated with increased risk of miscarriage. Use of pesticide combinations from different pesticide classes was also associated with increased risk of miscarriage. These findings are consistent with the present report. However, data also demonstrate a significant shift in the sex ratio of children borne to the spouse of appliers who applied fungicides and other products (Table 5); this is a new finding in the context of fungicide use and exposure.

Beginning in the 1960s, there has been a decline in the ratio of boy to girl children born, with a general decline in male birth predominance in the United States, Canada, and several European countries, including Italy, the United Kingdom, France, and Finland (James, 2000; Vartiainan et al., 1999; Davis et al., 1998; Astolfi & Zonta, 1999; Marcus et al., 1998; Allan et al., 1997). The magnitude of this fluctuation appears modest. For example, in the United States, a loss of 1 male birth per 1000 births between 1970 and 1990 was recorded (Allan et al., 1997). To account for these modest changes, investigators have focused their attention in several venues. Season of birth, birth order, ambient temperature, geographic latitude, and Darwinian survival factors including warfare and malnutrition were found to have an effect on the ratio of births of male to female children (Wells, 2000; Nonaka et al., 1990; Lerchl, 1999; Biggar et al., 1999; Anderson & Bergstrom, 1998; Graffelman & Hoekstra, 2000; Grech et al., 2000). Reports regarding altered sex ratio of children born subsequent to the Seveso incident and exposure to dioxins are more disturbing (Mocarelli et al., 2000). In those studies, a 35% reduction in number of male infants was noted. Similar findings have been recorded for children born to dibromochloropropane (DBCP) pesticide- and organochlorine pesticide-exposed workers (Davis et al., 1998). These environmental/occupational studies, including the present study, show significant reductions in male births in relatively small populations. The 25% reduction in male births in the children of applicators who apply herbicides, insecticides, and fungicides or among those applicators who apply mancozeb and/or triphenyltin fungicides is significant, but subject to question on biologic grounds.

Other laboratory-based human studies (Schrader et al., 2000), explored the possible relationship between exposure to triphenyltin and/or mancozeb in semen samples from pesticide applicators. Our preliminary results demonstrate that sperm morphology in these pesticide applicators was significantly altered and linked to chronic repeated seasonal exposure to these fungicides. Whether abnormalities of sperm morphology are factors in miscarriages or in the altered sex ratio of children borne to the spouse of the applicators of fungicides is uncertain (Lundin et al., 1997; Kobayashi et al., 1991). Data from in vitro studies utilizing MCF-7 cells, a breast cancer cell line, demonstrate that both mancozeb and triphenyltin will induce apoptotic cell death in relatively low concentrations (Lin & Garry, 2000). These data provide biologically plausible inferences regarding the reported increased miscarriage rate in the spouse of applicators of these fungicides.

Age at Menarche, Menstrual Irregularity, Menopause

Recorded in Figure 2 is the secular trend in the age of menarche by decade of birth. The pattern recorded here, namely, decreasing age at menarche of women borne in the years from 1920 through 1949, followed by a modest increase in the age of menarche in the years from 1950 through 1979, is not much different from data reported from northern Italy, Poland, and Denmark (Boldsen et al., 1993; Hulanicka & Walizko, 1991; Veroncsi & Gueresi, 1994). These data contrast reports from the southern United States, which suggest that continuing decreases in the age at menarche (Wattigney et al., 1999) may be due to increasing childhood obesity. Data from Michigan suggest that PCB exposure in utero and/or instant breastfeeding exposure to toxicant polychlorinated biphenyls can be correlated with earlier onset of menarche of these children (Blanck et al., 2000). The current trend of increasing age at menarche reported here is consistent with increased physical fitness activities among young women (Georgopoulos et al., 1999) or possibly, interference with the onset of menarche by a xenobiotic such as 2,4-D (Garry et al., 2001). The somewhat shorter reproductive life span (2.4 yr) in women living in a rural setting (Table 6) is not easily reconciled with the current published literature. A shorter reproductive life span has been reported among women with shorter menstrual cycles, among smokers, and among women with major depression (Harlow & Signorello, 2000). In our current study, the smoking rate among urban-born and rural women was no different; nor was there a significant difference in the frequency of menstrual-cycle irregularity between these two groups. A related topic of concern is the frequency of endometriosis. It is estimated that between 10 and 20% of women (NIH publication 91-413) of childbearing age have endometriosis. This data does not appear to be different from our current estimate (9%) of the frequency of endometriosis in our study group. None of the data discussed here, including age at menarche, menstrual cycle regularity, and endometriosis, is supportive of female-mediated endocrine disruptive effects in our study population. Data relating to significant differences in rural women's reproductive life span does not allow us to rule out endocrine-disruptive effects entirely. The occurrence of a high rate of miscarriages during the spring, the time of application of herbicides, also implies a female-mediated adverse reproductive event. However, the women's self-reported exposure assessment data derived from our survey do not support this contention.

Season, Miscarriages, and Pesticide Use

As indicated in Figure 1, over 10% of the pregnancies occurring in spring resulted in a miscarriage. Reports dealing with the seasonality of pregnancy loss and miscarriage in this country and in Europe, in general, acknowledge seasonal discrepancies in these adverse reproductive outcomes. Several studies report, with varying degrees of precision, that the miscarriage rate is highest in spring (Basso et al., 1995; Kallan & Enneking, 1992; Woodworth et al., 1994). Some parts of the reported seasonal variation are thought to be due to pregnancy planning bias, differences in fecundity, or perhaps some annually recurring environmental insult. In a contrasting U.S. national study by Warren et al. (1986), seasonal differences in the rate of miscarriages were not observed. These authors note that in earlier U.S. studies, rural areas in the north-central United States were overrepresented and speculated regarding potential geographic differences in the miscarriage rate. The present study demonstrates miscarriages occur most frequently among families whose male spouse applies herbicides in spring but does not apply fungicides later in the year. In earlier published works by members of our group (Garry et al., 1996b), it was demonstrated that conceptions in the spring led to significantly more birth defects among children of residents of the Red River Valley. Seasonal change in the birth defect rate was not observed for other geographically different regions of our state. Table 4 examines the pattern of spring herbicide use by applicators whose spouse had a first-trimester miscarriage in spring. Significant differences occurred in herbicide use patterns in pregnancies of the groups of subjects with and without miscarriages. For example, only 12.3% of the applicators used mixture 9100 (Cheyenne), but these accounted for 27.5% of the miscarriages occurring in spring. Of some interest in this connection, the product Cheyenne (a herbicide containing a combination of two sulfonylureas, one chlorophenoxy and one dibenzothiazole) has been in common use (beginning in 1991) for less than 10 yr (Meister, 1998). It is therefore temporally less likely that the herbicide mixture was a major contributor to the adverse reproductive outcomes observed. Five of the 11 spring miscarriages associated with applicator use of this product occurred after 1990, consistent with this suggestion. As a concern, one notes that there have been no animal reproductive toxicity studies of this herbicide mixture, though studies of individual components are available (Cheyenne FM Herbicide MSDS). It is also to be noted that the spouses of 7 applicators who applied Cheyenne had a total of 11 miscarriages in the spring. The seasonal recurrence of miscarriage in spring is puzzling and unrelated to confounding reproductive health risk factors such as multiple pregnancies, pelvic inflammatory disease, fertility drug treatment, and cervical os incompetence.

More pertinent to this discussion, the fungicides triphenyltin and mancozeb have been in common use for more than 20 yr to treat and protect wheat, potatoes, and sugar beets from fungal infestation. As mentioned earlier, these fungicides carry with them unresolved questions regarding human reproductive toxicity. Current published work from our laboratory shows that these fungicides can alter programming of cell death, an important developmental process, through induction of apoptosis in relatively low concentrations. Work in animals show that the major metabolite of mancozeb, ethylene dithiourea (ETU), is a potent disruptor of thyroid function and cause of thyroid cancer and is an animal teratogen (Hurley et al., 1998; Houeto et al., 1995). Triphenyltin is embryotoxic in animals (Noda et al., 1991; Ema et al., 2000). Altogether and individually, these fungicides demonstrate biologic plausibility as potential human reproductive toxicants. Those subjects (Tables 1 and 2) who applied herbicides in the spring, insecticides in the summer, and fungicides or fumigants in the fall had intermittent exposure to different toxicant classes. The categorical pesticide use data (Table 1) measured against the reproductive health endpoints examined also suggest the possibility of toxicant interaction and cumulative biologic toxicity during the application season. The reported use by acreage treated (Table 2) according to pesticide class, in part, lends some credence to this notion.

Finally, this study and other published works demonstrate the need to examine in greater detail male reproductive toxicity of antifungal agents. Human studies along these lines are underway. There is also a demonstrable need to examine the potential pharmacologic interactions of commercially available prepacked herbicide mixtures in animals for acute and reproductive toxicity. As the third and most important line of the effort, it is envisioned to develop and use prevention and control measures by working together with the community to develop sustainable environmental health practices in the Red River Valley.

REFERENCES

Allan, B. B., Brant, R., Seidel, J. E., and Jarrell, J. F. 1997. Declining sex ratios in Canada. *Can. Med. Assoc. J.* 156:37–41.

Anderson, R., and Bergstrom, S. 1998. Is maternal malnutrition associated with a low sex ratio at birth? *Hum. Biol.* 70:1101–1106.

Astolfi, P., and Zonta, L. A. 1999. Reduced male births in major Italian cities. *Hum. Reprod.* 14: 3116–3119.

Basso, O., Olsen, J., Bisanti, L., Juul, S., Boldsen, J., and the European Study Group on Infertility and Subfecundity. 1995. Are seasonal preferences in pregnancy planning a source of bias in studies of seasonal variation in reproductive outcomes. *Epidemiology* 6:520–524.

Biggar, R. J., Wohlfahrt, J., Westergaard, T., and Melbye, M. 1999. Sex ratios, family size and birth order. *Am. J. Epidemiol.* 150:957–962.

Blanck, H. M., Marcus, M., Tolbert, P. E., Rubin, C., Henderson, A. K., Hertzberg, V. S., Zhang, R. H., and Cameron, L. 2000. Age at menarche and tanner stage in girls exposed in utero and postnatally to polybrominated biphenyls. *Epidemiology* 11:641–647.

Boldsen, J. L., Jeune, B., Bach-Rasmussen, K. L., Sevelsted, M., and Vinther, E. 1993. Age at the menarche among schoolgirls in Odense. Is this still decreasing in Denmark? *Ugeskr. Laeger* 155: 482–484.

Davis, D. L., Gottlieb, M. B., and Stampnitzky, J. R. 1998. Reduced ratio of male to female births in several industrial countries. *J. Am. Med. Assoc.* 279:1018–1023.

Ema, M., Miyawaki, E., and Kawashima, K. 2000. Developmental toxicity of triphenyltin chloride in rats. *Teratology* 62:44A–45A.

Garry, V. F., Tarone, R. E., Long, L., Griffith, J., Kelly, J. T., and Burroughs, B. 1996a. Pesticide appliers with mixed pesticide exposure: G-banded analysis and possible relationship to non-Hodgkin's lymphoma. *Cancer Epidemiol. Biomarkers. Prevent.* 5:11–16.

Garry, V. F., Schreinemachers, D., Harkins, M. E., and Griffith, J. 1996b. Pesticide appliers, biocides and birth defects in rural Minnesota. *Environ. Health Perspect.* 104:394–399.

Garry, V. F., Tarone, R. E., Kirsch, I. R., Abdallah, J. M., Lombardi, D. P., Long, L. K., Burroughs, B. L., Barr, D. B., and Kesner, J. S. 2001. Biomarker correlations of urinary 2,4-D levels in foresters: genomic instability and endocrine disruption. *Environ. Health Perspect.* 109:495–500.

Georgopoulos, N., Markou, K., Theodoropoulou, A., Paraskevopoulou, P., Varaki, L., Kazantzi, Z., Lelise, M., and Vagenakis, A. G. 1999. Growth and pubertal development in elite female rhythmic gymnasts. *J. Clin. Endocrinol. Metab.* 84:4525–4530.

Gerhard, I., Monga, B., Waldbrenner, A., and Runnebaum, B. 1998. Heavy metals and fertility. *J. Toxicol. Eviron. Health A* 54:593–611.

Gold, E. B., and Tomich, E. 1994. Occupational hazards to fertility and pregnancy outcome. *Occup. Med. State-of-the-Art Rev.* 9:435–469.

Goldhaber, M. K., and Fireman, B. H. 1991. The fetal life table revisited: Spontaneous abortion rates in three Kaiser Permanente cohorts. *Epidemiology* 2:33–39.

Goldman, L. R. 1997. New approaches for assessing the etiology and risks of developmental abnormalities from chemical exposure. *Reprod. Toxicol.* 11:443–451.

Graffelman, J., and Hoekstra, R. F. 2000. A statistical analysis of the effect of warfare on the human secondary sex ratio. *Hum. Biol.* 72:433–445.

Grech, V., Vassallo-Agius, P., and Savona-Ventura, C. 2000. Declining male births with increasing geographic latitude in Europe. *J. Epidemiol. Commun. Health* 54:244–246.

Harlow, B. L., and Signorello, L. B. 2000. Factors associated with early menopause. *Maturitas* 35:3–9.

Houeto, P., Bindoula, G., and Hoffman, J. R. 1995. Ethylenebisdithiocarbamates and ethylenethiourea: possible human health hazards. *Environ. Health Perspect.* 103:568–573.

Hulanicka, B., and Walizko, A. 1991. Deceleration of age at menarche in Poland. *Ann. Hum. Biol.* 18:507–513.

Hurley, P. M., Hill, R. N., and Whiting, R. J. 1998. Mode of carcinogenic action of pesticides inducing thyroid follicular cell tumors in rodents. *Environ. Health Perspect.* 106:437–445.

James, W. H. 2000. Secular movements in sex ratios of adults and of births in populations during the past half-century. *Hum. Reprod.* 15:1178–1183.

Kallan J. E., and Enneking, E. A. 1992. Seasonal patterns of spontaneous abortion. *J. Biosoc. Sci.* 24:71–75.

Kavlock, R. J., and Setzer, R. W. 1996. The road to embryologically based dose-response models. *Environ. Health Perspect.* 104:107–121.

Kobayashi, T., Jinno, M., Sugimura, K., Nozawa, S., Sugiyama, T., and Iida, E. 1991. Sperm morphological assessment based on strict criteria and in-vitro fertilization outcome. *Hum. Reprod.* 7: 983–986.

Lerchl, A. 1999. Sex ratios at birth and environmental temperatures. *Naturwissenechaften* 86:340–342.

Lewis, V. 1997. The biology of early pregnancy loss. In *Loss during pregnancy or the newborn period,* eds. J. R. Woods, Jr., and J. L. E. Woods, pp. 37–69. Pitmam NJ: Jannetti.

Lin, N., and Garry, V. F. 2000. In vitro studies of cellular and molecular developmental toxicity of adjuvants, herbicides and fungicides commonly used in Red River Valley, Minnesota. *J. Toxicol. Environ. Health A* 60:423–439.

Lundin, K., Soderlund, B., and Hamberger, L. 1997. The relationship between sperm morphology and rates of fertilization, pregnancy and spontaneous abortion in an in-vitro fertilization/intracytoplasmic sperm injection programme. *Hum. Reprod.* 12:2676–2681.

Marcus, M., Kiely, J., Xu, F., McGeehin, M., Jackson, R., and Sinks. T. 1998. Changing sex ratios in the United States, 1969–1995. *Fertil. Steril.* 2:270–273.

Meister, R. T. (ed). 1998. *Farm chemicals handbook 1998.* Salem, MA: Meister.

Mocarelli, P., Gerthoux, P. M., Ferrari. E., Patterson, D. G., Kieszak, S. M., Brambilla, P., Vincoli, N., Signorini, S. Tracere, P., Carreri, V., Sampson, E. J., Turner, W. E., and Needham, L. L. 2000. Paternal concentrations of dioxin and sex ratio of offspring. *Lancet* 355:1858–1863.

Noda, T., Morita, S., Yamano, T., Shimizu, M., and Yamada, A. 1991. Effects of triphenyltin acetate on pregnancy in rats by oral administration. *Toxicol. Lett.* 56:207–212.

Nonaka, K., Desjardins, B., Charbonmeau, H., Legare, J., and Miura, T. 1990. Human sex ratio at birth and mother's birth season: multivariant analysis. *Hum. Biol.* 71:875–884.

Rempen, A. 1993. The abortion rate of viable pregnancies in the first trimester. *Zentralbl. Gynakol.* 115:249–257.

Savitz, D. A., Arbuckle, T., Kaczor, D., and Curtis, K. M. 1997. Male pesticide exposure and pregnancy outcome. *Am. J. Epidemiol.* 146:1025–1036.

Schrader, S. M., Turner, T. W., Shaw, P. B., Erickson, L. L., Holland, S., and Garry, V. F. 2000. Semen quality of men applying pesticides in northwest Minnesota. American Society of Andrology Meeting, April, abstr. 61.

Schreinemachers, D. M., Creason, J. P., and Garry, V. F. 1999. Cancer mortality in agricultural regions of Minnesota. *Environ. Health Perspect.* 107:205–210.

Scialli, A. R., Swan, S. H., Amler, R. W., Baird, D. D., Eskenazi, B., Gist, G., Hatch, M. C., Kesner, J. S., Lemasters, G. K., Marcus, M., Paul, M. E., Schulte, P., Taylor, Z., Wilcox, A. J., and Zahniser, C. 1997. Assessment of reproductive disorders and birth defects in communities near hazardous chemical sites. II. Female reproductive disorders. *Reprod. Toxicol.* 11:231–242.

Sever, L. E., Arbuckle, T. E., and Sweeney, A. 1997. Reproductive and developmental effects of occupational pesticide exposure: the epidemiologic evidence. *Occup. Med. State Art Rev.* 12:305–325.

Stellman, S. D., Stellman, J. M., and Sommer, J. F. 1988. Health and reproductive outcomes among American Legionnaires in relation to combat and herbicide exposure in Vietnam. *Environ. Res.* 47:150–174.

Vartiainen, T., Kartovaara, L., and Tuomisto, J. 1999. Environmental chemicals and changes in sex ratio: Analysis over 250 years in Finland. *Environ. Health Perspect.* 107:813–815.

Vassilev, Z. P., Robson, M. G., and Klotz, J. B. 2001. Associations of polycyclic organic matter in outdoor air with decreased birth weight: A pilot cross-sectional analysis. *J. Toxicol. Environ. Health A* 64:595–605.

Veronesi, F. M., and Gueresi, P. 1994. Trend in menarcheal age and socioeconomic influence in Bologna (Northern Italy). *Ann. Human Biol.* 21:187–196.

Warren, C. W., Gwinn, M. L., and Rubin, G. L. 1986. Seasonal variation in conception and various pregnancy outcomes. *Soc. Biol.* 33:116–126.

Wattigney, W. A., Srinivasan, S. R., Chen, W., Greenlund, K. J., and Berenson, G. S. 1999. Secular trend of earlier onset of menarche with increasing obesity in black and white girls: The Bogalusa Heart Study. *Ethn. Dis.* 9:181–189.

Wells, J. C. K. 2000. Natural selection and sex differences in morbidity and mortality in early life. *J. Theor. Biol.* 202:65–76.

Wilcox, A. J., Weinberg, C. R., O'Connor, J. F., Baird, D. D., Schlatterer, J. P., Canfield, R. E., Armstrong, E. G., and Nisula, B. C. 1988. Incidence of early pregnancy loss. *N. Engl. J. Med.* 319:189–194.

Woodworth, S. H., Singh, M., Sanfilippo, J. S., and Yussman, M. A. 1994. Seasonal effect on pregnancy loss rates in humans. *Ann. NY Acad. Sci.* 709:197–198.