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Male-mediated spontaneous abortion among spouses of stainless steel welders

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Objectives Male-mediated spontaneous abortion has never been documented for humans. The welding of stainless steel is associated with the pulmonary absorption of hexavalent chromium, which has genotoxic effects on germ cells in rodents. Clinical and early subclinical spontaneous abortions were examined among spouses of stainless-steel welders.

Methods A cohort of first-pregnancy planners was recruited from members of the union of metal workers and 3 other trade unions. The cohort was followed for 6 menstrual cycles from the cessation of contraceptive use. Altogether, 280 pregnancies were conceived, of which 35 were detected by human chorionic gonadotrophic hormone analysis and did not survive to a clinically recognized pregnancy. Information on exposure was collected prospectively in relation to the outcome and was available for all cycles resulting in a pregnancy. Information on pregnancy outcome was collected for all 245 clinically recognized pregnancies.

Results Increased risk of spontaneous abortion was found for pregnancies with exposure to paternal stainless-steel welding (adjusted relative risk 3.5, 95% confidence interval 1.3-9.1). The results were consistent in analyses of both biochemically and clinically recognized abortions. There was no increased risk for spontaneous abortion in pregnancies with paternal exposure to the welding of metals other than stainless steel.

Conclusions Male welding of stainless steel was associated with an increased risk of spontaneous abortion in spouses. A mutagenic effect of hexavalent chromium has been found previously in both somatic and germ cells, and the findings could be due to mutations in the male genome.

Key terms chromium, embryonal loss, follow-up study, male mediated, metals, mutagenicity, occupation, spontaneous abortion.

No documented case of male-mediated spontaneous abortion has been reported in humans (1, 2), but some facts suggest that the welding of stainless steel may be a relevant paternal exposure. First, fumes from the welding of stainless steel (SS), but not mild steel (MS), contain hexavalent chromium (3-5). Second, the welding of stainless steel may lead to pulmonary absorption of hexavalent chromium (4-7), and autopsy studies of former chromate-exposed workers have shown high concentrations of hexavalent chromium in all examined organs (8, 9). Third, animal experimental studies have demonstrated a mutagenic capability for hexavalent chromium in somatic cells and germ cells, and hexavalent chromium administered to a male rodent can impair the viability of embryos fathered by that male (1, 10-12). Fourth, human sperm with clinically documented genetic defects may be capable of fertilization and subsequently be responsible for reproductive or developmental defects in offspring, for example, Down's syndrome (13).

An increased risk of self-reported spontaneous abortion was previously found in spouses of stainless-steel welders, compared with spouses of nonwelders [odds

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ratio (OR) 2.0, 95% confidence interval (95% CI) 1.1-3.5] (14), but no excess risk was found in the same cohort in analyses based on abortions treated in a hospital (15), a finding which may be explained by high risk being restricted to early (preclinical) abortions.

The a priori defined aim of our study was therefore to see if male exposure to stainless-steel welding is associated with early spontaneous abortion. Information on exposure was collected before conception, and the information on spontaneous abortion included the biochemical detection of subclinical early pregnancy loss.

Subjects and methods

From 1992 to 1994, 430 couples were recruited for a time-to-pregnancy study after the nationwide mailing of a personal letter to 52 255 trade union members (metal workers, office and commercial workers, nurses, and day-care workers) who were 20-35 years old, living with a partner, and had no children. Only couples without previous reproductive experience who intended to discontinue contraception in order to become pregnant were eligible for enrollment. The couples were enrolled when they discontinued birth control, and they were followed for 6 menstrual cycles or until a pregnancy was recognized by the family practitioner. A detailed description of the study has been published elsewhere (16). The present study is based on pregnancies that occurred during the follow-up period of 6 menstrual cycles.

Assessment of exposure

At enrollment, both partners filled out a questionnaire on demographic, medical, reproductive, occupational, and life-style factors. During the follow-up, at day 21 in each menstrual cycle, the couples completed questionnaires on changes in occupational exposures and life-style factors. The men provided information on current and previous welding exposure, including type of metal (stainless steel, mild steel, other metal) and welding method, average daily duration of welding, and welding with or without application of local exhaust ventilation (17). On the basis of information on the menstrual cycle during which pregnancy was achieved, each pregnancy was categorized blinded to the outcome as exposed to (i) paternal stainless-steel welding, (ii) mild-steel, but not stainless-steel welding, or (iii) no welding. Nonmetal workers who reported welding were excluded, since no further information on welding was asked (N=24), leaving 406 couples for our study.

Detection of spontaneous abortion

Subclinical spontaneous abortions were detected in an analysis of urine samples during the time-to-pregnancy study. Samples were collected on 10 consecutive days from the onset of each period of vaginal bleeding and stored in home freezers. Urinary human chorionic gonadotrophic hormone (hCG) was measured in a time-resolved immunofluorometric assay for serum hCG (Delfia, Wallac, Finland). The method is specific for intact hCG and was validated for measurements of hCG in urine (16). In a negative reference group of 12 women who had not had intercourse during the previous 2 months, the urinary hCG was less than 0.5 IU/l in all the samples. All the samples from the first day of menstrual bleeding were analyzed for hCG, and, when the level was above 0.8 IU/l, all the other samples during the period were analyzed. When one of the values during a period was above 1 IU followed by a decline, the woman was regarded as having had an early pregnancy loss. The gestational length was calculated from recordings of menstrual bleeding in a diary the women kept during the study.

Information on the outcome of pregnancies that were clinically diagnosed during the 6 cycles of follow-up was obtained with a postal questionnaire in June 1996. Non-responding couples were interviewed by telephone, and a response rate of 100% was obtained. Questions were asked about date of termination of pregnancy and pregnancy outcome (live born child, stillbirth, spontaneous abortion, or induced abortion). The gestational length was calculated as the number of whole weeks from the first day of the last menstrual bleeding to the date of termination of the pregnancy.

Analysis and statistical methods

Spontaneous abortion was defined as fetal loss occurring up to 28 weeks of gestation. Survival curves were constructed by the Kaplan-Meier method (18). Rate ratios were calculated by Cox regression (SAS procedure PHREG), while controlling for potential confounding factors (18, 19). In 78 clinically recognized pregnancies, the women were not equipped with the kit for urinary sampling before the expected, but absent menstrual bleeding, and therefore these pregnancies were not at risk for the detection of an early pregnancy loss. The longest gestation among pregnancies terminated by an early pregnancy loss was 46 days, and the 78 pregnancies were included with left truncation on the 47th gestational day (18). Twenty-two women with a later documented early pregnancy loss became pregnant again in a subsequent cycle during the 6 cycles of follow-up. Because these observations need not be statistically independent, only the first pregnancy was included in the calculation of rate ratios. Crude risk measures were furthermore adjusted for the following potential confounding factors: center, female age, female body mass index, menstrual cycle length, male and female smoking, caffeine and

alcohol consumption, and reproductive disease. The proportional risk assumption was evaluated for all the variables by including time-dependent covariates and by comparing log-log survivor plots across strata (18). Possible interactions between welding and the confounding factors were tested by alternating the inclusion of product terms.

Results

The characteristics of the pregnancies are shown in tables 1 and 2. Altogether 280 pregnancies were detected among 258 women. Spontaneous abortion occurred in 71 pregnancies, of which 36 were clinically diagnosed and 35 were detected by hCG analysis and did not survive to

Table 1. Characteristics of 280 conceptions according to paternal exposure during the cycle of conception. (hCG = human chorionic gonadotrophic hormone)

	Type of exposure[a]					
	Stainless-steel welding[b]		Mild-steel welding[c]		No current welding	
	N	%	N	%	N	%
Demographic characteristics of the subjects	(N=23)		(N=54)		(N=203)	
Center						
Copenhagen	12	52	15	28	109	54
Aarhus	11	48	39	72	94	46
Female age						
≤24 years	13	57	21	39	71	35
25—29 years	10	43	29	54	119	59
≥30 years	-	0	4	7	13	6
Male age						
≤24 years	4	17	9	17	28	14
25—29 years	18	78	33	61	123	61
≥30 years	1	4	12	22	52	26
Mean menstrual cycle length						
≤24 days	1	4	3	6	1	1
25—34 days	20	87	39	78	163	84
≥35 days	2	9	8	16	30	16
Unknown	-	0	4	0	9	0
Female smoking (cigarettes/day)						
Nonsmoker	21	91	44	81	150	74
1—9 cigarettes/day	0	0	1	2	19	9
10—19 cigarettes/day	2	9	6	11	28	14
≥20 cigarettes/day	-	0	3	6	6	3
Male smoking (cigarettes/day)						
Nonsmoker	17	74	40	74	149	73
1—9 cigarettes/day	0	0	2	4	17	8
10—19 cigarettes/day	4	17	6	11	26	13
≥20 cigarettes/day	2	9	6	11	11	5
Clinically recognized pregnancies	(N=17)		(N=48)		(N=180)	
Gestational age at detection (weeks)						
≤5 weeks	7	41	17	36	99	56
6—7 weeks	7	41	19	40	53	30
≥8 weeks	3	18	11	23	26	15
Unknown	-	0	1	0	2	0
Pregnancy included in hCG protocol						
Yes, hCG kit was available	11	65	33	69	123	68
No[d]	6	35	15	31	57	32
Early pregnancy loss	(N=6)		(N=6)		(N=23)	
Onset of vaginal bleeding						
Days 26—30	1	17	3	50	6	26
Days 31—34	5	83	1	17	7	30
Days 35—46	-	0	2	33	10	43

[a] Total number of exposed: 23 in stainless-steel welding, 54 in mild-steel welding, 203 with no current welding.
[b] Twelve pregnancies were exposed to mild-steel welding as well. Stainless steel is an alloy of iron, nickel, and chromium, occasionally containing cobalt, vanadium, manganese, and molybdenum.
[c] Not exposed to stainless-steel welding. Mild steel is an alloy of iron, carbon, and silicon, occasionally containing molybdenum or manganese.
[d] Kit for sampling of first morning urine not delivered before the expected, but absent menstrual bleeding. Pregnancies included in analyses only after day 47.

Table 2. Male welding exposure in the cycle of conception.

	Stainless-steel welding[a] (N=23) (%)	Mild-steel welding[b] (N=54) (%)	No current welding (N=203) (%)
Welded metal[c]			
Mild steel	52	100	..
Stainless steel	100	0	..
Other	35	7	..
Welding method[c]			
MMA	57	41	..
MIG/MAG	48	87	..
TIG	91	15	..
Duration of stainless-steel welding			
None	0	100	..
≤1 hours/day	74	0	..
2—3 hours/day	13	0	..
≥4 hours/day	13	0	..
Protective measures[c]			
Local exhaust ventilation	74	48	..
Point exhaust ventilation	52	35	..
Air stream helmet	30	9	..
Any of above	91	67	..
Historical stainless-steel welding (years)			
None	0	74	92
≤1 years	15	12	6
2—5 years	45	6	2
≥6years	40	8	1

[a] Twelve pregnancies were exposed to mild-steel welding as well.
[b] Not exposed to stainless steel welding.
[c] MMA = manual metal arc, MIG/MAG = metal inactive or active gas, TIG = tungsten inert gas. The categories are not mutually exclusive.

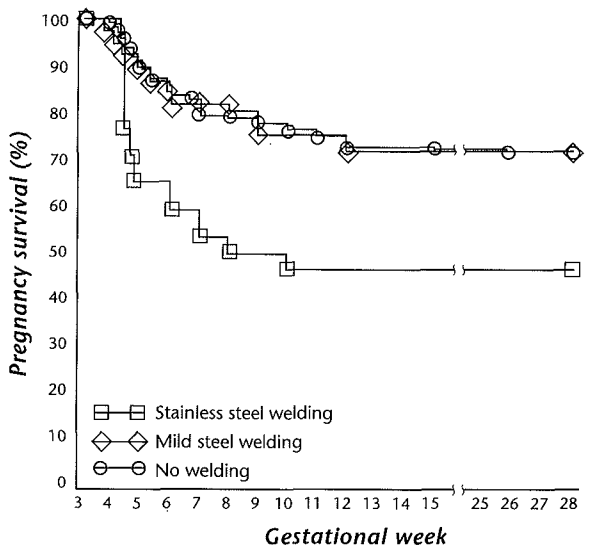

Figure 1. Kaplan-Meier survival curve for pregnancies (N=280) according to paternal welding.

a clinically detected pregnancy (table 1). During 202 pregnancies in which the women were equipped with the kit for urinary sampling, 17.3% and 12.4% of the pregnancies were terminated by an early pregnancy loss and a loss of a clinically recognized pregnancy, respectively, resulting in a total risk of spontaneous abortion of 30.2%. There were no induced abortions.

Pregnancy survival was lowest for pregnancies with paternal exposure to stainless-steel welding (figure 1). The relative risk (RR) for spontaneous abortion was increased in a comparison with pregnancies without paternal welding exposure (RR 2.6, 95% CI 1.2-5.5). No elevated risk was found for pregnancies exposed to mild-steel welding (table 3). The results were robust to adjustment for potential confounding factors (table 3). No statistically significant interactions were found between stainless-steel welding and any of the confounders. The estimated risk was still significant in subanalyses in which the reference group was divided into spouses of nonwelding metal workers (N=52) and nonmetal workers (N=132), and in separate analyses of early pregnancy loss and loss of clinically recognized pregnancies (RR 3.0, 95% CI 1.1-8.0, and RR 3.2, 95% CI 1.1-9.8, respectively). Excluding the left truncated pregnancies did not change these results, and the proportional hazard assumption was not violated for any variable. However, all spontaneous abortions in spouses of the stainless-steel welders took place before the 10th gestational week.

The risk of pregnancy loss increased as the number of years of stainless-steel welding increased. The relative risks for pregnancy loss with 1 to 5 years and >5 years of stainless-steel-welding exposure were 1.2 (95% CI 0.4-3.3) and 2.6 (95% CI 1.1-6.1), respectively, compared with no historical paternal stainless-steel-welding exposure (table 2). The estimates were adjusted for female age. The number of observations did not allow for further analyses of the modifying effect on stainless-steel welding by, for example, welding method, intermittence, and protective measures (table 2).

Discussion

Our findings indicate an increased risk of early spontaneous abortion for women whose partners are engaged in stainless-steel welding during the cycle in which the woman conceived. Welding of mild steel, which does not involve exposure to hexavalent chromium, was not associated with an increased risk.

Sources of bias

The result is unlikely to be explained by selection bias because none of the couples had previously experienced a pregnancy, even less a spontaneous abortion, and information on pregnancy outcome was obtained for each of the 245 clinically recognized pregnancies. Pregnancy recognition bias could have played a role if the exposed subjects recognized their pregnancies earlier than the unexposed did (20), but this was not the case in our study (table 1). Furthermore, the proportion of pregnancies included in the hCG protocol was similar among the 3

Table 3. Pregnancy loss according to paternal exposure to welding — frequency and relative risk with 95% confidence interval.

Paternal welding exposure	Pregnancies	Spontaneous abortion		Crude relative risk[a]	95% CI[b]	Adjusted relative risk[c]	95% CI[d]
		N	%				
No welding	203	48	23.6	1	.	1	.
Mild-steel welding[e]	54	13	24.1	1.1	0.6—2.1	1	0.5—2.1
Stainless-steel welding	23	10	43.5	2.6	1.2—5.5	3.5	1.3—9.1

[a] Risk estimates were calculated for the 1st pregnancy of each woman (N=258 pregnancies).
[b] For the crude relative risk.
[c] Adjusted for center, female age, female body mass index, menstrual cycle length, male and female smoking, caffeine and alcohol consumption, and reproductive disease.
[d] For the adjusted relative risk.
[e] Not including pregnancies exposed to paternal stainless-steel welding.

exposure groups. Information on exposure was collected prior to pregnancy outcome for all pregnancies, and therefore recall bias was unlikely.

Coherence with other studies

In 2 other studies, the proportion of early pregnancy loss was 22% and 13%, respectively (21, 22), which is within the range of our finding of 17.3%. The national average frequency of hospitalized spontaneous abortion is about 9% (23), close to our average of 9.6% of spontaneous abortions that occurred after 8 gestational weeks.

In our cohort of 406 couples, time to pregnancy was not statistically significantly increased for the couples exposed to stainless-steel welding. The odds ratio for conception per cycle was 0.8 for stainless-steel and mild-steel welders and 0.9 for other welders in comparison with nonwelding metal workers (17). It could be expected that a substantially increased risk of early pregnancy loss would result in an increase in the time needed to obtain a clinically recognized pregnancy. However, an incidence of early pregnancy loss such as we observed leads to an expected odds ratio of 0.7 for conception per cycle for women with a stainless-steel-welding partner; this value is close to the observed odds ratio of 0.8.

Evaluation of the results

Toxicokinetic and autopsy studies have indicated a very slow elimination of chromium from some compartments of the body, and this phenomenon may result in a gradual build-up of chromium in some tissues during long-term exposure (7-9, 24). Unfortunately no studies have investigated a possible accumulation in the testes. Our data suggest that long-term exposure may be as relevant a risk indicator as current exposure. The biological significance of this finding is to some degree corroborated by a previous finding of an increase in chromatid breaks in lymphocytes associated with cumulated stainless-steel-welding exposure (3).

Different mechanisms have been suggested for male-mediated effects on offspring (1). The germ cell may be directly affected by genetic or epigenetic mechanisms or indirectly by the transmission of agents to the mother (eg, via the seminal fluid). Analyses of semen provided by males included in the present study showed no statistically significant differences attributable to welding in terms of sperm density, morphologically abnormal sperm, or sperm motility, as assessed by computer-assisted sperm analysis (25). Thus a possibly responsible mechanism for the findings is not reflected in the traditional parameters of semen quality.

In conclusion, our a priori hypothesis of an increased risk of early spontaneous abortion in spouses of stainless-steel welders was corroborated. Similar results were obtained in analyses using biochemical and self-reported information on pregnancy loss. Although the number of exposed pregnancies was limited, the results were consistent, and the prospective collection of data on exposure in relation to the outcome makes it unlikely that the finding is due to chance or uncontrolled bias.

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