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Exposure to Leather Tanning Factories Lowers Semen Quality in Mongolian Men

Purevtogtokh Myagmartseren[1], Alan Geater[2], Hutcha Sriplung[2]

[1]Department of Infertility and Reproductive, National Center for Maternal and Child Health (NCMCH), Ulaanbaatar, Mongolia; [2]Epidemiology Unit, Faculty of Medicine, Prince of Songkla University, Songkhla, Thailand

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Corresponding Author
Purevtogtokh Myagmartseren, MD, MSc
National Center for Maternal and Child Health (NCMCH), Khuvisgalchid Street, Ulaanbaatar 16060, Mongolia.
Tel: +976-11-362886
E-mail: pujess@yahoo.com

Objectives: To examine the difference in semen qualities of male workers in leather tanning factories and of those in other industries. **Methods:** Eighty reproductive aged male workers from six leather tanning factories and eighty-five from non-tanning workplaces participated in the study as the exposed subjects and non-exposed subjects, respectively. Each subject was given a physical examination, required to complete a questionnaire, and asked to supply a semen specimen. Semen analysis on fresh specimens followed the latest World Health Organization guideline. Semen parameters were compared between the exposed and non-exposed subjects using tabulation and chi-square test for categorical variables and t-test or rank-sum test for continuous variables. Multiple linear or logistic regression models were constructed to examine the independent effects of exposure. **Results:** After adjusting for confounding variables, exposed subjects had significantly higher percentages of grade C motility (p=0.008), mid-piece defect (p=0.009), and tail-defect (p=0.029) in their spermatozoa compared to non-exposed subjects. Liquefaction time was also longer (p=0.046). **Conclusion:** Leather tanning workers have significantly higher percentages of grade C motility, mid-piece defect, and tail-defect in their spermatozoa.

Keywords: Tanning, Male, Reproductive Health, Semen Quality

Introduction

Modern tanning technology involves the use of chromium and organic solvents as well as many other chemicals [1-3]. Leather tanning workers have been shown to have elevated levels of chromium in their body fluids, and male workers to have poorer reproductive health [4-12]. This effect might be attributable to their exposure to chromium, as chromium, particularly hexavalent chromium, has been shown to have an adverse effect on semen quality. Welders exposed to hexavalent chromium have been found to have decreased sperm concentration and motility in some but not all studies [13,14]. Semen analysis from trivalent chromium sulphate production workers have also been reported to have a significantly higher proportion of abnormal spermatozoa [15].

Leather tanning mostly involves trivalent chromium, but

under certain conditions, the chromium of tanned hides can convert to the hexavalent form [16,17]. Thus, handlers of chromium tanned hides may be exposed to not only trivalent but also to hexavalent chromium. Earlier studies in Mongolia reported that the fertility of leather tanning workers may be lower that of the general population [10,12]. Both studies used questionnaire and urine samples to reveal reproductive health status of leather tanning workers. Exposure to organic solvents has also been reported to have an adverse effect on male fertility by decreasing sperm concentration and motility [8,13,18,19].

Leather production is a major industry in Mongolia. According to 2010 statistics, Mongolia has 32.7 million livestock and in 2007 was reported to have processed 7.5 million hides, with an even higher marketing capacity of 10 million [20]. Lack of effective regulatory system in the industry results in a hazardous working environment for workers. To meet market demand, tanning factories are constantly increasing their capacity, leading to deterioration in working conditions, which is likely to entail greater exposure to hazardous chemicals. The purpose of this study was to estimate the magnitude of adverse effect on male semen quality of exposure to leather production work and identify which parameters of semen quality are compromised.

Materials and Methods

1. Study design and populations

This cross-sectional study was conducted in Ulaanbaatar, the capital city of Mongolia, and approved by the ethics committees of the Maternal and Child Health Research Center, Ulaanbaatar, and Prince of Songkla University, Thailand. The semen quality of male workers in leather factories was compared with that of controls working in other types of factories or in the military. All workplaces were located in the same administrative areas, and thereby all subjects were exposed to similar environments outside their particular workplace.

Ten leather tanning factories in Ulaanbaatar were asked to participate in this study, among which six agreed to participate as collection sites for exposed subjects. All male workers between the ages of 18 and 45 years from the six factories were invited to participate, from which a total of 90 workers agreed (response rate = 23.5%). Three non-leather factories (bakery, cashmere, furniture) and one military unit were asked and agreed to participate as collection sites for non-exposed subjects, from which 94 workers agreed to participate.

Consecutive sampling of both exposed and non-exposed subjects was based on meeting the inclusion criteria of being willing to participate and aged between 18 and 45 years. In addition, exposed subjects had to have worked in a leather tanning factory for at least 3 months, and non-exposed subjects had to have never worked in a leather tanning factory.

The researcher held one-on-one meetings with each potential participant to determine eligibility. An interview and physical examination were conducted by the researcher to ensure that he had a healthy medical history, no history of sexually transmitted disease, and no evidence of genital abnormality. Participants who failed to meet the eligibility requirements were excluded from the study.

Eligible participants were asked to provide written consent and fill out a comprehensive questionnaire on working conditions and personal habits, number of years in the current occupation, type of job, hours of work, use of personal protective equipment, and frequency of alcohol consumption and of tobacco use. They were also requested to provide a urine specimen and a semen specimen by masturbation after 2 to 7 days of abstinence from sexual activity. Upon submission of these specimens, days of sexual absence was recorded.

In the end, the sample was reduced to 80 exposed subjects and 85 non-exposed subjects. Nineteen subjects were lost from the analysis due to the following reasons: 10 subjects provided only a urine sample, 7 subjects submitted their semen sample too late for analysis, and the specimens of 2 subjects were spilled.

2. Semen analysis

At each collection site, a mobile lab was set up to analyze semen samples, which had to be analyzed within one hour to be included in the analysis. Subjects were asked to masturbate at home or at the workplace to supply a semen sample. Based on the subject's timing for abstinence days, we scheduled an appointment with each subject to collect the semen sample. Fresh samples were kept at 38°C to determine the time to liquefaction. Analysis was conducted within half an hour after delivery for 65% of subjects. Each specimen was well mixed, and its volume was recorded before examining with phase-contrast microscopy at 200x–400x. Following the WHO latest guideline, the parameters assessed were motility, vitality, and morphology, based on counts of 200 spermatozoa [20]. Concentration was

also assessed.

To assess motility, a 10 µl sample of semen was transferred using an "Eppendorf" micropipette to a microscope slide and covered lightly with a cover slip. Two hundred spermatozoa were graded as progressive (grades A and B), non-progressive (grade C), and immotile (grade D) types of motility. To assess vitality, semen samples were dyed with eosin-nigrosin. Air-dried smears of the dyed semen specimens were then stained using the Papanicolaou method to assess sperm morphology.

To estimate sperm concentration, 50µl samples of the semen were diluted 1:20 with a standard solution. The diluted sample was transferred to a Neubauer haemocytometer to count the number of spermatozoa in each chamber. If the difference between the two chambers of the haemocytometer count exceeded the 95% of confidence interval, a recount was conducted. Total sperm count calculation was based on actual concentration of spermatozoa multiplied by the volume of semen.

All semen analyses were performed by one laboratory technician. A random, external quality test of 8% of the samples resulted in discrepancies of less than 10% for counts of motility, morphology, and concentration.

3. Assessment of chromium

Graphite furnace atomic absorption spectrophotometer (GFAAS) (Shimadzu AA-650 1F) was used to measure chromium concentrations in the urine of a subset of participants. Furnace temperature was set at 2400°C. Initial calibration was performed with distilled water and with standard solutions of 0.25%, 0.5%, 1%, 2%, 4% chromium. All urine samples were kept frozen until testing, then after being thawed at room temperature, 2cm^3 of each sample was dropped into the specimen tube of the GFAAS.

4. Statistical analysis

The sample size calculation to detect a difference in the mean values of the semen parameters between the two groups were based a power of 80%, at an alpha of 0.05, with a minimum of half standard deviation. Allowing for 10% unusable samples, the final minimum sample size required was 63/0.9 = 70 from each group.

Socio demographic, personal habit, and semen specimen characteristics were compared between exposed and non-exposed groups, and differences were tested for statistical significance using chi-square, Fisher's exact, or t- or Mann-Whitney tests, as appropriate. Some right-skewed continuous variables were logarithm transformed to render their distributions more symmetrical.

Associations between each semen parameter and leather tanning factory work were explored through a series of linear regression models in which age, years in current occupation, direct occupational exposure to chemicals, and number of days since last sexual encounter were initially included as covariates that could be potential confounders, together with any other variables shown in the univariate analysis to indicate some association with the semen parameter. The level of statistical significance was set at 0.05.

Results

Parameters of socio demographic and personal habit characteristics of subjects in each group are shown in Table 1. Compared to non-exposed subjects, exposed subjects were slightly older, had less education, and were more likely to use personal protective equipment. However, no significant differences were found in body status (height, weight, or BMI), alcohol consumption, and tobacco use frequency (Table 1).

Chromium concentration was assessed in only 31 exposed subjects and 10 non-exposed subjects. Median (IQR) of chromium concentration in urine was 1.65 ppm (0.33, 4.79) and 0.3 ppm (0.02, 3.30) respectively, as shown in Table 1.

Crude values of semen parameters of the two groups are shown in Table 2. Median volume of semen among exposed subjects was 2.2 cm^3, compared with 2.8 cm^3 among non-exposed subjects (p=0.098).

Spermatozoa with progressive motility (grades A and B) accounted for a mean of 55.4% (SD 6.1) of spermatozoa in exposed subjects and 57.6% (SD 5.9) in non-exposed subjects (p=0.017). Exposed subjects had a higher proportion of grade C spermatozoa (median 12.4%, IQR 8.9,16.4) than non-exposed subjects (median 9.6%, IQR 7.1,12.2) (p=0.001).

The proportion of spermatozoa with mid-piece defect were higher in exposed subjects (mean 20.0%, SD 6.0) than non-exposed subjects (mean 15.7%, SD 6.2) (p<0.001). Additionally, the promotion of spermatozoa with tail defect were higher in exposed subjects (median 5.1%, IQR 3.0, 9.8), than non-exposed subjects (median 3.8, IQR 2.4, 5.9) (p=0.004). By contrast, the

Table 1. Socio demographic, personal habit characteristics and chromium concentrations of exposed and non-exposed subjects.

Characteristic/Concentration	Exposed subjects (n=80) Mean (±SD)	Non-exposed subjects (n=85) Mean (±SD)	p-value
Age (years) [mean (SD)]	27.77	24.82	0.010 [a]
Work duration [median (IQR)]	2 (0.6,3)	0.6 (0.6,3)	0.330 [b]
Height (cm) [mean (SD)]	170 (6.8)	171 (5.7)	0.311 [a]
Weight (kg) [mean (SD)]	66.65(9.063)	67.39 (7.622)	0.311 [a]
BMI kg m^{-2} [mean (SD)]	23.09 (2.973)	23.01(1.911)	0.453 [b]
Marital status [no.(%)]			0.128 [c]
Married	50 (62.5%)	42 (49.4%)	
Single	28 (35.0%)	4 (49.4%)	
Divorced	2 (2.5%)	1 (1.2%)	
Education [no.(%)]			<0.001 [b]
Low	6 (7.5%)	5 (5.9%)	
Medium	68 (85%)	56 (65.9%)	
High	6 (7.5%)	24 (28.3%)	
Use of personal protective equipment [no.(%)]			<0.001 [d]
Yes	78 (97.5%)	50 (58.8%)	
No	2 (2.5%)	35 (41.2%)	
Alcohol consumption [no.(%)]			0.188 [d]
Do not drink	36 (42.9%)	48 (57.1%)	
Do drink	44 (54.3%)	37 (45.7%)	
Cigarettes smoked per day[no.(%)]			0.085 [d]
Do not smoke	25(45.5%)	30 (54.5%)	
Up to 5	18(36.7%)	31 (63.3%)	
Up to 10	28 (59.6%)	19 (40.4%)	
Up to 20	9 (64.3%)	5 (35.7%)	
Urinary chromium (IQR) (ppm)	1.65(0.33, 4.79)	0.3(0.02, 3.30)	0.010 [b]

[a] t-test; [b]Wilcoxon rank sum test; [c]Fisher's exact test; [d] Chi-square test.

proportion of spermatozoa with head defect was higher in non-exposed subjects (46.6%, SD 9.0) than in exposed subjects (41.5%, SD 6.9) (p<0.001). Median sperm concentration was $40.6x10^6$ cm^{-3} (IQR: 30.9 $x10^6$, 57.4 $x10^6$) among exposed subjects and $46x10^6$ cm^{-3} (IQR: 30.9 $x10^6$, 81.8 $x10^6$) among non-exposed subjects (p=0.059).

Using the definition of normal semen from the WHO guideline, 37.5% of exposed subjects had normal semen compared with 50.6% of non-exposed subjects (p=0.09); the other subjects had at least one parameter below WHO standards (Table 3). However, the percentages of subjects having one or more parameters below the WHO standard were not significantly different between the two groups.

Table 4 summarizes the semen parameters of the two groups using a multiple linear regression analysis. The multiple linear regression models are adjusted for age, years in current occupation, direct occupational exposure to chemicals, and number of days since last sexual encounter. According to the models, exposed subjects had a 1.22 time (95% CI 1.05, 1.40) higher percentage of grade C motility (p=0.008), an increased percentage (+2.93%; 95% CI 0.73, 5.13) of spermatozoa with mid-piece defect (p=0.009), and a 1.35 times (95% CI 1.03, 1.76) higher percentage of spermatozoa with tail-defect than non-exposed subjects. Mean liquefaction time was 1.76 (95% CI 0.03, 3.48) minutes longer (p=0.046). However, exposed subjects had a decreased percentage (-3.77%; 95% CI -6.71,

Table 2. Semen parameters of exposed and non-exposed subjects

Semen parameter	Exposed subjects (n=80)	Non-exposed subjects (n=85)	p-value
Volume [median (IQR)]	2.2 cm^3 (1.0, 4.4)	2.8 cm^3 (1.2, 5.0)	0.098[b]
Motility (%)			
A+B [mean (SD)]	55.4 (6.1)	57.6 (5.9)	0.017[a]
C [median (IQR)]	12.4 (8.9, 16.4)	9.6 (7.2, 12.2)	<0.001[b]
D [mean (SD)]	31.6 (6.4)	32.1 (5.3)	0.593[a]
Vitality (%)[mean (SD)]	68.5 (6.3)	68.2 (5.6)	0.671[a]
Sperm morphology (%)			
Normal [mean (SD)]	31.2 (7.0)	32.9 (6.7)	0.117
Head defect[mean (SD)]	41.5 (6.9)	46.6 (9.0)	<0.001[a]
Mid-piece defect [mean (SD)]	20.0 (6.0)	15.7 (6.2)	<0.001[a]
Tail defect [median (IQR)]	5.1 (3.0, 9.8)	3.8 (2.4, 5.9)	0.004[b]
Concentration (x 10^{-6})[median (IQR)]	40.6 (30.9, 57.4)	46 (30.9, 81.8)	0.059[b]
Total sperm count (x10^6) [median (IQR)]	95.6 (67.2, 147.2)	122 (77.0, 226.8)	0.023[b]

[a] t-test; [b]Wilcoxon rank sum test

Table 3. Selected semen parameters compared to the WHO standard

Semen parameter	Exposed subjects No. (%)	Non-exposed subjects No. (%)	p-value*
Normal semen	30 (37.5%)	43 (50.6%)	0.09
At least one value < normal WHO	50 (62.5%)	42 (49.4%)	0.125
<2 ml	20 (25%)	16 (18.8%)	0.440
Vitality less than 60%	7 (8.8%)	4 (4.7%)	0.466
<50 % progressive motility (A+B)	16 (20%)	9 (10.6%)	0.142
<20x10^6 concentration	5 (6.2%)	1 (1.2%)	0.109[c]
<30% morphology	34 (42.5%)	30 (35.3%)	0.430

* [c]Fisher's exact test

Table 4. Multiple linear regression analysis on semen parameters of exposed and non-exposed subjects.

Semen parameter	Measure	Exposed subjects	Non-exposed subjects	Adjusted models* (Exposed vs. Non-exposed Subjects)		
				Discrepancy	95% CI	p-value
Grade C motility (%)	Geometric mean (GSD)	12.3 (1.5)	9.7 (1.5)	x 1.22	(1.05, 1.40)	0.008
Liquefaction time (min)	Mean (SD)	32.9 (5.0)	31.7 (4.6)	+ 1.76	(0.03, 3.48)	0.046
Head defect (%)	Mean (SD)	41.5 (6.9)	46.6 (9.0)	- 3.77	(-6.71, -0.84)	0.012
Mid-piece defect (%)	Mean (SD)	20.0 (6.0)	15.7 (6.2)	+ 2.93	(0.73, 5.13)	0.009
Tail defect (%)	Geometric mean (GSD)	5.4 (2.2)	3.7 (2.1)	x 1.35	(1.03, 1.76)	0.029

SD Standard deviation; GSD Geometric standard deviation

-0.84) of spermatozoa with head-defect (p=0.012). Other dependent variables, including alcohol consumption, tobacco use frequency, and testis size, had no detectable association with any of the semen parameters examined. The adjusted R-squared value of the multiple regression model was 61%.

Adjusted for: age, years engaged in current occupation, direct occupational exposure to chemicals and number of days since last sexual encounter

Discussion

The study revealed increased proportions of spermatozoa with mid-piece defect and with tail defect, as well as a higher proportion of grade C motility, in men working in leather tanning factories compared to those working in other industries.

A previous study revealed that leather tanning workers were 5.6 times more likely to experience infertility [10]. Sperm movement and concentration can be a good predictor of fecundity [15]. This study found that sperm defects were significantly increased and sperm motility and concentration were decreased in leather tanning workers, which is consistent with above mentioned study. Over 60% of exposed subjects in our study were classified as having abnormal semen, compared with only 49% of non-exposed subjects.

Various occupational and semen analysis studies have reported conflicting results [22]. Two reproductive studies reported that the morphology and concentration of sperm was poorer in welders than in controls [13, 14]. However, another study failed to find any difference in the semen parameters between steel welders and controls [23]. This study has shown that liquefaction time has significantly longer in leather tanning workers; some studies have shown prolonged time among welders, while other studies have shown longer times among controls [13, 22].

Our study was unable to determine if the lower quality of semen parameters among leather tanning factory workers was due to exposure to organic solvents or the endocrine-disrupting effects of various chemicals used in the leather tanning process [3, 8, 13, 24, 25]. Despite 97.5% of the leather tanning workers reporting using personal protective equipment, the disposal masks and homemade aprons they used are of questionable protective effectiveness. Wet working conditions and cold temperatures during winter months can have a negative effect on the genital-urinary system of workers. Leather tanning is an undesirable job in Mongolia because of low wages and poor working environment, thus disadvantaged people often do such work in Mongolia; this difference could have biased the study findings.

Our study has some methodological advantages over the previously conducted studies in Mongolia. Most importantly, our study examined both semen parameters and urine chromium while the previous similar studies examined only the urine sample [10,12]. One limitation of our study was that only 23.5% of leather tanning factory workers agreed to participate in the study, which may perhaps indicate a difference between those who agreed to participate and those who did not agree. Those who previously suspected some health problems may have volunteered more readily than those with no suspected problems. However, the same may have been true of the controls. A second limitation of our study stems from the use of one semen specimen from each participant. To satisfy the WHO standard of a fertility investigation, a minimum of 2 specimens are required. However, despite these limitations, the results of our study warrant further investigation of a well-organized cohort of workers to confirm or refute the apparent harmful effect on semen quality of men working in the leather tanning industry.

In conclusion, our study has revealed evidence of an association between increased percentages of mid-piece defect and tail defect in the spermatozoa, as well as increased percentages of grade C motility, among those who work in the leather tanning industry. The agent(s) responsible remain to be elucidated.

Conflict of Interest

The authors declare that there were no competing interests related to this study.

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