Effect of Tannery Work Exposure on Seminal Fluid Parameters in Iraqi Men

Alaa N. Mohammed Ali, Samal H. Kareem, Noor AL-Huda A. Abdul Hussein, Ali I. Mohammed
Department of Biology- College of Science- Al-Mustatansiriyah University - Baghdad, IRAQ

Abstract
Background:
Tannery work share exposure to solvents and chemicals, which has been linked with inverse pregnancy and fertility outcomes in human being as reported in some studies.

Objective:
The current study aimed to through some light on the harmful pollutant chemicals effect on tannery workers reproductive health. Literature in this portion are rare in Iraq, so it had been tested the hypotheses that leather workers suffered from male infertility parameters due to the continuous exposure to chemicals that used in this industry.

Subjects, Materials and Methods:
This study included 60 subjects can divided into 2 groups: 30 males as study group and 30 males as control group, the period of study was between October 2014 to April 2016, the samples were taken from tanneries workers from various governorates of Iraq.

Results:
The data clearly showed a significant infertility parameters among these workers represented by low total sperm number, low sperm activity the significance levels were within the value of P ≤0.05 accompanied with a significant high seminal fructose.

Conclusion:
The main conclusion was that chronic exposure to these chemicals in tannery workers especially Cr VI and N,N-dimethyle formamide may cause male infertility parameters.

Key words: Tannery workers, male infertility, sperm number, sperm activity, seminal fructose, Cr VI and N,N-dimethyle formamide.
Introduction:

Rising the global indigence for leather has forced the growth of the leather tanning industry in modern decades with plenty of expansion occur in developing countries (1), leather work has been involved as a risk factor for several diseases these include nasal, para-nasal, bladder, lung and testicular cancer (2,3). There is also some guide of a connection between leatherwork and haematopoietic malignancies, although this is believed to be related to insinuation to solvents rather than leather itself (4,5).

Leather tannery workers may exposed to diversity of chemicals which usually include trivalent chromium (Cr III), ammonia, calcium hydroxide, hydrogen sulfide, sulfuric, hydro chronic acid (1,6,7), N,N-dimethyl formamide (8) and the carcinogenic hexavalent chromium (Cr VI) which had also been used (9). Chromium is a metallic element that occur in a diversity of oxidized states, trivalent +3 (Cr III) and hexavalent +6 (Cr VI) which is very toxic (10). Towering chromium levels blood, urine and body tissues are existed in workers occupationally exposed to Cr (11), workers who are exposed to chromium in welding suffered from increasing risk of reduced semen quality, sperm abnormality leading to infertility, the seminal fluid analysis which remains the cornerstone of the infertility evaluation (12). Occupational exposure to (Cr VI) led to lowering sperm number, sperm motility and show essential abnormalities in the tissue of the testicles (13). Testicular tissues of (Cr VI) administrated rats showed powerful degeneration and clearly suggested that Cr VI sub acute treatment causes oxidative stress in rat testes leading to apoptosis (14), Greene et al. also mentioned the chronic (Cr VI) administration at sub lethal dose decreased weight of secondary sex organs and epididymis sperm number, which set an interference with spermatogenesis (Cr VI) and lead to minimizing sperm motility, protein phosphorylation, mitochondrial membrane potential and metabolic enzyme activity and it suggested that chromium affects boar sperm motility by break tyrosine phosphorylation in the mid piece of sperm by cutting the cAMP/PKA pathway in boar sperm in vitro (16). There was clear allocation by (Cr VI) in the germ cell population in the seminiferous tubule with degenerative
changes in Leydig cells\textsuperscript{15}. Des \textit{et al.} study showed that (Cr VI) is cytotoxic and could destroy the physiological functions of male somatic cells (Leydig cells and Sertoli cells) and spermatogonial, damage or dysfunction of these cells can directly affect spermatogenesis, resulting in male infertility\textsuperscript{17}. Oxidative stress induced by CrVI was cytotoxic to both male somatic cells and spermatogonial induced mitochondria-dependent apoptosis.

N, N- dimethyl formamide (DMF) has been used widely in the industry (synthetic leather, resin and fiber polyurethen polyacrylic etc...) exposure to DMF was associated with liver toxicity, and alcohol intolerance \textsuperscript{8}.

The prostate and seminal vesicles weight, sperm motility and normal morphology were significantly reduced in swiss CD1 mice developing toxicity with DMF \textsuperscript{18,19}. Chang \textit{et al.} reported that exposure to DMF could minimize the sperm speed, limiting capacity and beat frequency of sperm. So exposure to DMF can lead to hypoactivity in sperm \textsuperscript{21}.

Other many studies have reported the carcinogenic, skin and respiratory effect of occupation in tanneries. Reproductive health effect have not been discussed widely. There appear to be no studies that addressed reproductive outcomes in male tannery workers in Iraq as far as it known, so the aim of this study was to investigate the harmful effect of chronic exposure to chemical material used in synthetic leather industry in Iraqi male tannery workers.

\textbf{Materials and Methods :}

This study was carried out in private laboratory in Baghdad through the period from October 2014 till April 2016 on 30 male tannery workers (study group), and 30 healthy males (as control group). Samples were taken from tanneries workers from various governorates of Iraq who came to be examined in Baghdad. The chemical
materials that used in leather industry in Iraq tanneries workshops in Baghdad, these chemical materials are used tanneries in Iraq according to local leather tanneries, which include: \{\{ NaCL, Sodium Sulfide, Ca(OH)2, H2So4, Formic acids (CHCO2H), Sodium Bicarbonate, Trivalent Chromium (Cr III), Hexavalent Chromium (Cr VI), Iso Cyanide, Polyol, Methyl Chloride, Chlorine – Ethan Soil, Silicon Oil, N,N-dimethyl formamide (DMF), Poly Vinyl Chloride (PVC) \}\}.

**Data collection :-**

Sixty males were involved in this study and divided into groups first one as study group which included thirty males aged between 20 – 45 years were included in this study as workers in leather industry who were married and did not have children for a period of one year {Had unprotected regular coitus for at least one year}. Those patients had no infection, painful deformities of infertility.

The information was obtained regarding work history, medical history, and drinking alcohol, none of the subjects were reported liver and adrenal gland disorders (Questionnaire form was designed especially for this study). The Seminal fluid were collected from these patients (through masturbation) in polystyrene sterile jars after a period of no sex (abstinence period) ranged from (3 – 5 days).

These study standard lines were also taken into consideration with the healthy subjects as control group which included also thirty males their age ranged between 22 – 47 years. The Macroscopic and Microscopic examination procedures were done according to recommendation of the World Health Organization (22), seminal fluid volume, sperm concentration and motility grades were assessed. The microscopic examination was done using a fully automatic digitalization process using Computer Assisted Semen Analysis (CASA) (22). Acidosis measured by Pocket pH meter by HANNA® instrument USA. Fructose in semen was measured by Semen Assay® Fructose - Kit for Determination of the Fructose Level in Seminal Plasma.

**Statistical analysis :**

Results are expressed as mean ± standard error (M±SE). Considered data that has been analyzed by one-way analysis of variance (ANOVA) followed by fisher’s test for multiple comparison using Stat View software.
version 5.0. The differences were considered significant when P value ≤ 0.05. Regression analyses were conducted through covariance analysis (ANCOVA) for correlation also by using Stat View software version 5.0.

**Results:**

This study was obviously presented the wide aberration that appeared in tannery workers. This study was clearly showed a significant (p≤ 0.05) decrease in sperm concentration (Total sperm Number (TSN)) , sperm motility as compared with control group as showed in table (1) . Total sperm activity (TSA) or total sperm motility also decreased significantly (P ≤ 0.05) in study group compared with control group (table (1)).

Also there was a respectable increase (p≤ 0.05) in semen fructose in study group compared with control group as showed in table (2) . There was no significant variation in semen pH and semen volume between control and study group.

While a negative significant correlation (P ≤0.05) was detected in study group between total sperm number and fructose levels as showed in figure (1), also a negative significant correlation (P ≤0.05) appeared between total sperm activity and fructose levels in study group as shown in figure (2) , this correlation was not appeared in control group as shown in figures (3 and 4).

**Table (1): Certain Sperm Parameters between control group and study group (Tannery workers).**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Classification</th>
<th>TSN (Million/ml)</th>
<th>GA % (M±SE)</th>
<th>GB % (M±SE)</th>
<th>GC % (M±SE)</th>
<th>GD % (M±SE)</th>
<th>TSA % (M±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study</td>
<td></td>
<td>*39.697 ± 4.299</td>
<td>*4.147 ± 0.777</td>
<td>*4.147 ± 0.777</td>
<td>*6.209 ± 0.787</td>
<td>*6.209 ± 0.787</td>
<td>*16.138 ± 1.739</td>
</tr>
</tbody>
</table>

TN S= Total Sperm Number or Sperm Concentration (Million/ml).
GA = Grade A (means sperm moves ahead quickly).
GB = Grade B (means sperm move ahead slowly or sluggish).
GC = Grade C (means sperm doesn’t move ahead).
GD = Grade D (means sperm is still).
TSA = Total Sperm Activity or Total Sperm Motility =GA+GB+GC (M±SE)= Mean± standard error
* = Significant Difference P≤ 0.05.
Table (2): Seminal Fluid, pH and Seminal Plasma Fructose level between control group and study group (Tannery workers)

<table>
<thead>
<tr>
<th>Classification</th>
<th>Volume (ml) (M±SE)</th>
<th>pH (M±SE)</th>
<th>Fructose (mmol/L) (M±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>3.125 ± 0.229 NS</td>
<td>7.500 ± 0.035 NS</td>
<td>*3.757 ± 0.288</td>
</tr>
<tr>
<td>Study</td>
<td>2.076 ± 0.118 NS</td>
<td>7.468 ± 0.048 NS</td>
<td>*9.020 ± 0.412</td>
</tr>
</tbody>
</table>

(M±SE)= Mean ± standard error
* = Significant P ≤ 0.05 .
NS = Non Significant.

Figure (1): shows the significant correlation P≤ 0.05 between fructose level and TSN (Total Sperm Number) million/ml in study group, R =0.475.
Figure (2): shows the significant correlation between fructose and TSA {Total Sperm Activity} % in study group, R= 0.489.

Figure (3): shows the non-significant correlation P≤ 0.05 between fructose level and TSN {Total Sperm Number} million/ml, in control group, R= 0.135.
Discussion:

Although leather workers are in secure to many chemical substances, but most of them are toxic or sub toxic and can cause many anomalies, which affect fertility, specially Cr VI and DMF \(^{(23,20,24)}\).

The data clearly showed a significant \((P \leq 0.05)\) effect of total sperm number, total sperm activity in tannery workers (study group), this may be caused by Cr VI.

Elevated levels of chromium in blood, urine and some body tissues are found in workers exposed to chromium \(^{(11)}\), the workers who exposed to chromium were suffered from increasing risk of minimize semen quality, sperm defects lead to infertility \(^{(12)}\), many studies shown that Cr VI is a major risk factor to growing testicles and cause testicular atrophy \(^{(25)}\), decreased sperm count and high mortality in adult mice \(^{(26)}\), another study also mentioned that Cr VI motivate dysfunction in male reproduction organs \(^{(24,27)}\).

Testicular tissues of Cr VI administered rats showed powerful degeneration, these results clearly suggest that Cr VI subacute treatment causes oxidative...
stress in rat testes leading to apoptosis\textsuperscript{(14)}.

The study concluded that the significant decreases in total sperm number is due to toxic chronic exposure to Cr VI that may affect testicular tissue of these workers.

Present study showed an significant $P \leq 0.05$ decreases of total sperm activity ,this low activity may be due to DMF. Chang \textit{et al.} found that workers in a direct exposition to DMF have the worst behavior of sperm motility\textsuperscript{(20)} ,workers who have been occupationally exposed to DMF could be at risk of disturbance of motility of sperm especially in velocity beating amplitude and beating frequency\textsuperscript{(21)}.

The physiological cause of sperm low activity is due to low mitochondrial function in sperm. Exposure of DMF reduced mitochondrial activity in the sperm, mitochondrial function is one of the causal factors that is identified for causing decreased sperm motility\textsuperscript{(20)}, the mitochondria of sperm located in the middle piece provide the necessary power flagella wave\textsuperscript{(20)},the male infertility can result from a respectable lowering in the numbers of motile sperms or from movement quality disorder\textsuperscript{(28)}.

However exposure to DMF is associated with negative effects of sperm mitochondria is not understood at the present time\textsuperscript{(20)}.

Shieh \textit{et al.} suggested that DMF increases oxidative stress through slashing the natural antioxidant, glutathione, and drain the power of cell power houses, by registering their capacity to use oxygen and glucose to produce energy thereby lowering the ability of the dopamine-producing cells. Cr VI can minimize the sperm motility, protein phosphorylation, mitochondrial membrane potential and metabolic enzyme activity. It suggested that chromium can affect the boar sperm motility by break tyrosine phosphorylation in the mid piece of sperm by cutting the cAMP/PKA pathway in boar sperm \textit{in vitro}\textsuperscript{(16)}.

Sudamani \textit{et al.} reported that one of the first studies on streptozotocin in mice model for diagnosis of diabetes which suggested that high blood glucose has led to reduction of prostatic acid phosphatase concentration and increasing fructose level in semen. Insulin treatment of diabetic mice restored seminal performance to control levels, on the other hand, chronic exposure with Cr VI effect on the ultra-structure of
pancreas islets and liver\textsuperscript{(30)}. So it effect the glucose homeostasis, and therefore the metal induce modification of pancreas and liver may caused by elevated sugar level in the blood, these evidence may explain the high fructose levels in semen\textsuperscript{(31)} which were found in study results.

Data correlation in study group (tannery workers) showed a negative significant correlation $P \leq 0.05$ between total sperm number with semen fructose (Figure 1) and between total sperm activity with semen fructose levels in tannery workers (Figure 2).

It is of interest that the proportional weights of seminal vesicles and prostate were higher in rats exposed to chromium compared to untreated animals. Moreover, the histological view of seminal vesicle and prostate of chromium treated animals presents enlarged areas filled with secretions\textsuperscript{(32)}. This could be caused by intermediates increase reactive of inflammation which was related to chromium VI toxicity\textsuperscript{(33)}. This abnormality caused high fructose secretion.

Amidu \textit{et al.} reported that fructose appeared with attachment negatively with sperm count and decreased motility, this study observed that the significantly higher seminal fructose concentration were in azoospermia and oligospermia patients\textsuperscript{(34)}. This study was relevant to study of Manivannan \textit{et al.} study who confirmed the defect in the normal function of the seminal vesicles.

In this study there was no significant differences in pH between two groups (control group and tannery workers group), increasing fructose lead to high pH due to consumption of fructose by the sperms as a source of energy\textsuperscript{(36)}, but in this study it believed that the low numbers and low activity of sperms lead to low consumption of fructose (low lactic acid production) leading to non elevated pH level even with high fructose level (low sperm activity with low pH). So fructose is an important source of energy for the sperm, and, thus the concentration of fructose in the semen, all of which can change as a result of decomposition of fructose, the main source of lactic acid in the semen\textsuperscript{(37)}. 
References:


20. Chang, H-Y.; Shih, T-S.; Guo, Y-S.; Guo, Y-L.; Tsai, C-Ti. And Hsu, P-CH. Sperm function in workers exposed to N,N-dimethylformamide in the synthetic leather


37. Mann, T. and Lutwak, Mann, C. Male reproductive function and