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Occupational Hazard as a Risk Factor for Azoospermia among Infertile Men

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Abstract

Objectives: Exposure to environmental contaminants is a major risk factor for overall human health, including fertility. There has been increasing evidence of association of male infertility with occupational hazards such as heat, chemicals, and radiation. This study aimed to evaluate if certain job engagements and the environment have an impact on seminal characteristics of infertile men. **Methods:** 327 infertile men engaged in different occupations were divided into two groups: Group 1, who had a high likelihood of being exposed to occupational hazards; and Group 2, whose occupations had less or no hazardous working environment. Semen analysis was performed and the accessory gland function was also evaluated. **Results:** The farmers outnumbered those from other occupations (102/327). We observed a significantly higher incidence of azoospermia cases (16/39) among factory workers and a two-fold higher odds ratio in Group 1 (OR: 0.27, 95% CI: 0.184, 0.41) compared to Group 2 (OR: 0.14, 95% CI: 0.083, 0.239). Differences in semen parameters such as semen volume, pH, total sperm count, and sperm of normal morphology between the two groups were found to be statistically significant. Construction workers recorded the lowest semen volume and the highest seminal pH, while police personnel and factory workers had the least total sperm count and sperm with normal morphology. **Conclusions:** This study indicates an association of certain occupations with male infertility. Therefore, it is recommended to take precautionary measures to minimize exposure to workplace-related environmental hazards.

Keywords: Azoospermia, Male Infertility, Occupational Hazards, Semen Parameter, Total Fertility Rate

1. Introduction

With the dramatic increase in environmental contaminants during the recent years, male infertility is also rising rapidly. Studies have revealed a decline in the quality of human semen over the last few decades, and occupational and environmental hazards cause impairment of spermatogenesis, leading to male infertility. The first ever report on workplace hazards impacting reproductive function appeared in 1970, when a high impotence rate was reported among farmers working with pesticides[1]. It was believed to be the effect of the pesticide DDT (Dichlorodiphenyltrichloroethane) causing impotence by lowering the level of the hormone testosterone[2]. In 1975, a reduction in the quality of semen was reported among workers occupationally exposed to lead[3]. Another study highlighted impairment of spermatogenesis caused by the pesticide Dibromochloropropane, DCBP, among pesticide plant workers who are males[4]. An analysis of 101 studies published between1934-1996 found a significant decline in average sperm density among men of highly industrialized Western countries. On the contrary, this trend was not observed in men of non-Western nations for which only limited data was available[5]. Surprisingly, in 2002, an estimated 186 million couples were reported to be infertile in developing countries, excluding, China according to an World Health Organization report[6]. This might have been the result of rapid industrialization and

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extensive application of harmful chemical pesticides, fertilizers, etc., in agricultural practices in developing countries including India. Poor management of waste disposal from industries, factories and agriculture is also a leading cause of air, water, and soil contamination. Moreover, some of the chemical substances that are banned in the Western countries are still in use in some of the developing countries.

During the past decade, many studies have reported the impact of environmental pollutants on overall health, including fertility, in humans. India being one of the most polluted countries in the world its fertility rate is declining among its population in an unprecedented manner. For the first time, the Total Fertility Rate (TFR) of Indians has fallen below the replacement level, i.e., the ability to produce the population for the next generation, according to the National Family Health Survey report 2019-21. The national TFR has been reduced to 2.1 in rural areas, and 1.6 in urban areas with an average of 2.0. The state of Karnataka, one of the leading industrial states in India, recorded an average TFR of only 1.7. The southern districts of Karnataka are comprised of both urban, and rural areas, with agriculture as the primary occupation in most of the rural areas, while urban areas have heavy industries, manufacturing units, and factories with white and blue collar jobs. In order to understand the impact of various environmental hazards, individuals who are exposed to these hazards in higher concentrations in their occupational setting would be better study-subjects rather than the general population. So far, there has been no report of studies conducted in Karnataka state which examined the reproductive potential of men affected by occupational exposure. Taking this note, certain job descriptions may require the workers to be exposed to occupational hazards like heat, chemicals and radiation that may pose a risk to reproductive function. Therefore, this study was conducted to assess the association of different occupations with male factor infertility by semen examination and evaluation of male accessory gland function tests.

2. Materials and methods

2.1 Study Population

The study was conducted after approval by the Institutional Human Ethical Committee, University of Mysore, Mysore, India (IHEC-UOM No.143/Ph.D/2016-17). A total of 327 infertile men aged 25-50 years from 10 districts of South Karnataka seeking fertility treatment were taken as subjects in this study. The exclusion criteria while recruiting the study subjects were infertile men with a history of medical conditions like diabetes, hypertension, cryptorchism, tubule defects, STDs, hepatitis, genetic or chromosomal abnormalities, etc. The patient's data, including age, occupation, and work environment, along with their reproductive histories, were recorded in a well-designed questionnaire. Expressed consent was obtained from each participant. The patients were divided into two groups depending on their occupation and likelihood of having exposure to certain environmental hazards that may have an impact on fertility, as shown in Table 1. Farmers (n=102), drivers (n=28), factory workers (n=39) and construction workers (n=20) were combined in Group 1 (N=189) because these occupations are at high risk of being exposed to heat, chemical and radiation hazards. Group 2 (N=138) included police (n=9), businessmen (n=87), office workers (n=30) and electricians (n=12), all of whom had a lower risk of occupational hazards. For purpose of convenience, automobile, tobacco, and tyre manufacturing workers were included in factory workers; construction workers included builders, welders, painters, carpenters; and teachers; accountants, software engineers, employees of bank, railways, universities, and private companies were grouped under office workers.

2.2 Semen Analysis

The patients were advised to maintain a minimum of 2-3 days of sexual abstinence before semen sampling. Semen was evaluated for its macroscopic and microscopic properties following the WHO Guidelines for Examination and Processing of Human Semen, 5th edition. Macroscopic semen parameters included volume, liquefaction time, and pH. Sperm concentration, total sperm count, sperm motility, and sperm morphology were the microscopic parameters.

2.3 Determination of Fructose in Semen

Fructose is secreted by the seminal vesicles, and it is the main source of energy for sperm motility. Twenty micro-litres of seminal plasma were diluted with 220 µL of distilled water, then 50 µL of $ZnSO_4$ and 50 µL of NaOH and incubated for 15 minutes. After centrifugation at 2500 rpm, 200 µL of the supernatant was mixed with 200 µL of Indole reagent, 2 mL of 32% HCl and incubated for 20 minutes at 60°C. The absorbance was read at 470 nm and the concentration of fructose was calculated.

2.4 Determination of Citric Acid Level in Semen

Citric acid, a biomarker for prostate gland function, helps to maintain the pH of the semen and its liquefaction time. Ten micro-litres of seminal plasma were mixed with 10 µL of 50% TCA (Trichloro acetic acid) and centrifuged at 7000 rpm for 15 minutes. To the supernatant, 800 µL of acetic anhydride was added and incubated at 60°C for 40 mins. After cooling for 5 min, the absorbance was read at 400 nm in a spectrophotometer.

2.5 Statistical Analysis

The categorical variables were expressed as frequency (n) and percentage (%) and the numerical variables as means and standard deviations. For comparison of the frequency distribution of different male infertility phenotypes in Group 1 and Group 2, *p* values were derived from the Chi square test. We calculated the odds ratio and 95% confidence interval separately for the two groups and also for the overall patients among the different infertility phenotypic groups. Further, the risk estimate analysis was performed for all the different occupations for azoospermia cases. Lastly, the independent test was the preferred method in the analysis of semen parameters, fructose and citric acid levels among the groups. A *p*-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using the IBM SPSS Statistics 20.

3. Results

3.1 Frequency of infertile groups

The average age of the patients was 35.10 ± 5.96 years (mean ±SD). Semen samples from all 327 patients were analysed. In 174 patients, normal semen parameter or idiopathic cases were observed; 64 patients had no spermatozoa in semen or non-obstructive azoospermia; 10 patients had a reduced number of sperm (<15 million per mL) i.e., oligospermia; 38 patients had less than 40% of progressively and non-progressively motile sperms or asthenospermia; 35 patients showed both oligo- and asthenospermia and all dead sperm in semen or necrospermia were observed in 6 patients.

In Table 1, the frequency of infertile male patients attending to different occupations is shown. An over-representation of farmers (n=102) in Group 1 and an under-representation of police (n=9) in Group 2 was observed. Further, we found a statistically significant higher proportion of azoospermia cases in Group 1 (n=45; 70.31%) as compared to Group 2 (n=19; 29.69%). Also, the frequency of oligospermia cases was significantly lower in Group 1 (n=2; 20%) than in Group 2 (n=8; 80%).

{N=Total; n=frequency; %=percentage, *OAs= Oligoasthenospermia)

3.2 Risk Estimate

Table 1 reveals that there is a significant over-representation of azoospermia cases in Group 1 and a significant under-representation of oligospermia cases. So, we calculated the odds ratio and 95% confidence interval of different infertility phenotypes in Group 1 and Group 2 patients as shown in Table 2. The result showed significantly two-fold increase in the proportion of azoospermia cases again in Group 1 (OR:0.275; 95% CI:0.184-0.41) compared to Group 2 (OR:0.14; 95% CI:0.083, 0.239) while oligospermia cases had a six- fold decrease in odds ratio value (OR:0.009; 95% CI: 0.002-0.054) in Group 1 compared to Group 2 (OR:0.054; 95% CI:0.026-0.114).

Further, we plotted a forest plot for all the different infertility groups for overall patients considered in the study which revealed the highest odds ratio value for azoospermia as shown in Figure 1. When the odds ratio was calculated for the overall population in the eight different occupational groups, factory workers had the highest proportion of azoospermia cases compared to all the other occupations (Figure 2).

3.3 Sperm Parameters

Semen parameters and the levels of male accessory gland secretions of all the eight different occupational groups in Groups 1 and 2 were separately estimated as shown in Table 3. Further, statistical analysis including all the occupational subgroups combined into Group 1 who had a higher likelihood of exposure to occupational hazards and Group 2 who were less likely to be exposed to occupational hazards was performed to compare for any significant difference in their values between the two groups (Table 4). The result showed a significant

Table 1. Frequency distribution of different infertility phenotypes in relation to men in different occupations included in the study

Infertile groups (N=327)	Group 1 (N=189)				Group 2 (N=138)				P value
	Factory workers (n=39) (%)	Farmers (n=102) (%)	Construction workers (n=20) (%)	Drivers (n=28) (%)	Policemen (n=9) (%)	Businessmen (n=87) (%)	Office workers (n=30) (%)	Electricians (n=12) (%)	
Idiopathic, n N=174	17 (43.59%)	46 (45.10%)	18 (90%)	18 (64.29%)	3 (33.33%)	48 (55.17%)	14 (46.67%)	10 (83.33%)	0.725
Azoospermia, n N=64	16 (41.03%)	22 (21.57%)	1 (5%)	6 (21.42%)	2 (22.22%)	13 (14.94%)	2 (6.67%)	2 (16.67%)	0.024*
Oligospermia, n N=10	0 (0%)	2 (1.96%)	0 (0%)	0 (0%)	0 (0%)	6 (6.90%)	2 (6.67%)	0 (0%)	0.014*
Asthenospermia, n N=42	2 (5.13%)	20 (19.61%)	0 (0%)	0 (0%)	4 (44.44%)	12 (13.79%)	4 (13.33%)	0 (0%)	0.990
O.As, n N=31	4 (10.26%)	10 (9.80%)	1 (5%)	4 (14.29%)	0 (0%)	6 (6.90%)	6 (20%)	0 (0%)	0.656
Necrospermia, n N=6	0 (0%)	2 (1.96%)	0 (0%)	0 (0%)	0 (0%)	2 (2.30%)	2 (6.67%)	0 (0%)	0.221

Table 2. Odds Ratio and 95% Confidence interval of infertility groups in Group 1 and Group 2

Infertility phenotypes	**OR (95% Confidence interval)**	
	Group 1	**Group 2**
Idiopathic	0.967 (0.676, 1.385)	1.047 (0.702, 1.56)
Azoospermia	0.275 (0.184, 0.41)	0.14 (0.083, 0.239)
Oligospermia	0.009 (0.002, 0.054)	0.054 (0.026, 0.114)
Asthenospermia	0.116 (0.071, 0.149)	0.149 (0.088, 0.251)
Oligoasthenospermia	0.098 (0.058, 0.084)	0.084 (0.045, 0.157)
Necrospermia	0.009 (0.002, 0.026)	0.026 (0.009, 0.073)

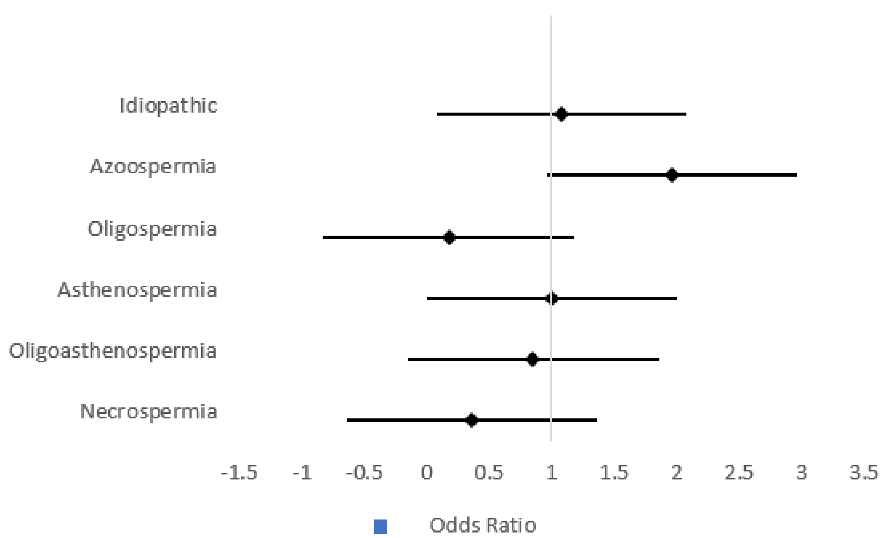

Figure 1. Forest plot depicting the overall Odds Ratio and 95 % CI of different infertile phenotypes in the study.

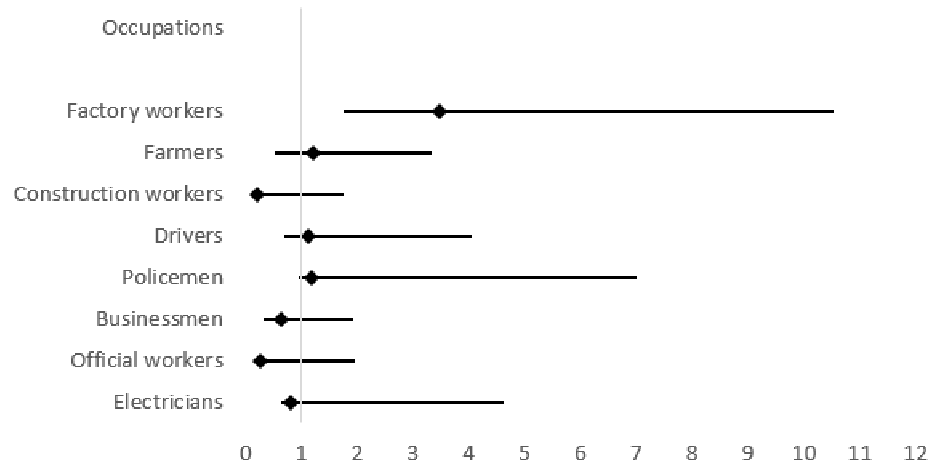

Figure 2. Forest plot derived from Odds ratio and 95 % CI of different occupations of men having Azoospermia.

Table 3. The average semen parameters and accessory gland secretion levels for different occupational sub groups in Group 1 and Group 2

Semen parameters	Group 1				Group 2			
	Factory workers	Farmers	Construction workers	Drivers	Policemen	Businessmen	Office workers	Electricians
Volume (mL)	2.06±1.48	1.65±1.07	1.61±0.93	1.91±0.77	2.16±0.90	1.87±1.28	2.35±0.99	2.58±0.56
Liquefaction time (min)	32.82±10.05	40.09±25.61	34.75±1.91	34.11±16.99	28.44±3.08	38.85±22.95	40.33±17.01	31.66±7.78
pH	7.87±0.36	7.86±0.35	8.0±0.28	7.71±0.31	7.72±0.50	7.74±0.4	7.83±035	7.7±0.37
Sperm concentration (10^6/mL)	38.07±45.64	32.42±31.85	51.8±27.47	36.3±31.74	28.22±39.73	35.77±31.44	41.81±33.14	60.91±46.79
Total sperm count (10^6/ ej)	86.79±137.93	52.16±78.65	83.2±94.42	66.79±63.76	48.55±61.56	73.86±94.82	107.51±119.45	168.79±148.91
Progressively motile sperm (%)	18.46±22.09	15.54±18.51	23.0±17.5	20.5±16.43	19.11±34.66	20.36±20.76	21±17.32	27.21±21.82
Normal sperm morphology (%)	29.97±26.25	46.95±28.15	57.80±23.72	42.60±26.44	49.33±33.30	48.43±26.41	57.86±21.55	50.16±30.79
Accessory gland function test								
Fructose (mg/ej)	48.07±44.89	50.55±51.01	71.52±65.01	65.6±62.42	28.81±22.10	47.5±52.06	56.41±41.72	45.54±39.8
Citric acid (μmol/ej)	48.54±43.97	51.77±51.22	41.42±27.36	53.16±36.24	16.36±6.51	65.97±99.62	81.18±109.84	35.14±43.92

Table 4. Comparison of semen parameters and accessory gland secretions between Group 1 and Group 2

Sperm parameters	Group 1	Group 2	p value
Volume (mL)	1.77±1.12	2.05±1.169	**0.025***
Liquefaction time (min)	37.14±21.15	37.86±20.21	0.755
pH	7.85±0.34	7.75±0.39	**0.015***
Sperm concentration (10^6/mL)	36.21±34.95	38.77±34.34	0.510
Total sperm count (10^6/ ej)	64.76±94.22	87.78±107.39	**0.041***
Progressively motile sperm (%)	17.67±18.95	21.00±21.12	0.136
Normal sperm morphology (%)	43.95±28.08	50.69±26.30	**0.028***
Accessory gland function test			
Fructose (mg/ej)	54.49±53.47	48.05±47.61	0.261
Citric acid (µmol/ej)	50.22±45.56	63.37±96.08	0.101

*ej: ejaculate

decrease in the average semen volume among men in Group 1 (mean±SD; 1.77±1.12), construction workers being the lowest as compared to Group 2 (mean±SD; 2.05±1.169) patients' semen samples **(**p=0.025***)**. We also found significantly higher pH in Group1 (mean±SD: 7.85±0.34) than in Group 2 (mean±SD 7.75±0.39) with a p-value of 0.015***.** Construction workers again reported the highest pH of 8.0±0.28 compared with men in other occupations. A statistically significant (p=0.041*) decline in the average total sperm count was also observed in Group 1 (64.76±94.22) when compared to Group 2 (87.78±107.39)**,** while the lowest total sperm count was observed in patients with police as occupation (48.55±61.56). Examination of the percentage of sperm with normal morphology revealed significantly lower mean and standard deviation values of 43.95±28.08 in Group 1 and 50.69±26.30 in Group 2 (p=0.028*). Interestingly, factory workers represented the occupation with the lowest proportion of morphologically normal sperm (29.97±26.25). No significant difference was found in the other parameters considered in the study.

4. Discussion

Studies on the impact of occupational hazards on male infertility are scarce and even conflicting. Innumerable chemical compounds are synthesized to manufacture products that improve our modern lifestyle. But these compounds have affected human health and fertility in a frequency- and dose-dependent manner. However, due to the multifactorial nature of male infertility, it appears impossible to evaluate the effect of a single occupational hazard on the fertility potential in men. Therefore, we studied the association of male infertility with different occupations by semen examination and evaluating male accessory gland function.

A study suggested that manual workers are at a higher risk of having male infertility than non-manual workers[7]. Therefore, the individuals who performed manual work in their occupational setting were combined together in Group 1 and those occupations that required the use of intellect rather than physical strength in Group 2. Job descriptions included in Group 1 were factory workers, farmers, drivers, and construction workers; and business, police, office workers, and electricians were combined in Group 2. In our study, the frequency of farmers was overrepresented and had the lowest proportion of progressively motile sperm in the semen compared to other occupations among infertile patients. Farmers are prone to chemical exposure through the routine use of chemical pesticides. Pesticides are mainly organophosphorous and organochlorine compounds, with DDT (Dichlorodiphenyltrichloroethane) and DBCP (Dibromochloropropane) being the most commonly used ones[8]. Pesticide application has been dramatically increased in the agricultural practices to feed the ever-growing world population. But some of the pesticides are endocrine disrupters, and impair sperm production in men by causing sperm DNA damage when exposed to higher concentrations[9].

The group with azoospermia, or complete absence of sperm in semen, was found to be in the highest proportion, with a two-fold higher odds ratio value in Group 1 when compared to Group 2. The factory workers, as their job description, scored the highest odds ratio for the proportion of azoospermia among all the other occupations included in the study. Also, semen analysis data revealed the lowest percentage of sperm with normal morphology among them. Factory workers have a high exposure to physical and chemical toxins such as extreme heat, phthalates, and dioxins compound[10]. Most of the factory workers recruited in our study were automobile-, tyre manufacturing-, and tobacco processing workers. Workers in automobile manufacturing industries are subjected to excessive heat, vibrations, chemical compounds, heavy metals like lead, and radiation exposure[11]. The effect of non-ionizing radiation on the reproductive health of men is still not evident, but ionizing radiation causes chromosomal alteration[8]. In the tyre factory, workers are exposed to a wide variety of chemical compounds like nitrosamines, aromatic hydrocarbons, solvents, phthalates, sulfur, etc., as well as dust and fume inhalation[12]. Tobacco processing factories produce toxic chemicals like ammonia, nicotine, nitrates, methanol, and hydrochloric acid[13].

Our study also showed a significant decline in average semen volume, and an increase in seminal pH among the Group 1 occupational group. Construction workers' seminal volume and pH were found to be significantly lower and higher, respectively. A person in this occupation may be exposed to physical and chemical hazards such as high temperature and toxic organic solvents[14]. Exposure to excess heat leads to abnormal semen characteristic phenotypes like azoospermia, oligospermia, and teratozoospermia[15]. Organic solvents are known to cause damage to testis in experimental animals, and exposure to lead causes a decline in sperm production and prostate function[10,16]. The secretions from the prostate gland aid in maintenance of pH and secretions from both the seminal vesicles and prostate gland contribute to seminal volume. But, no statistical significance could be observed in their biomarkers, i.e., citric acid and fructose, in our study. Formaldehyde, widely used in construction and wood processing units, has adverse effects on sperm motility[17].

We observed a significant difference in total sperm count between the two groups. Group 1 had lower total sperm count compared to Group 2. But, the individuals who had police as their occupation showed the lowest total sperm density or count. This may be due to the excessive exposure to air pollution. Several studies have reported that prolonged exposure to air pollutants like sulfur dioxide, carbon monoxide, nitrogen dioxide and particulate matter has the ability to negatively impact sperm density, morphology and motility, thereby decreasing fertility potential and sperm quality in men[18].

A major drawback of this study is the lack of measurement of any potential hazard like chemical compounds but complete reliance on information available in questionnaire for exposure assessment and the presence of cofounding variables like lifestyle factors. However, our study has shown that certain occupational settings have higher likelihood of causing infertility in men. In conclusion, we suggest maintenance of strict safety standard measures in the workplaces and educating the workers of the potential risk involved in the type of work is necessary to prevent high level exposure to the health hazards.

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