

## **Semen Analysis in Fertiliser Factory Workers**

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### **ABSTRACT**

Study case-control has been conducted to analyse the relationship of occupational exposure in fertilizer plant with semen analysis. The case is the exposed and control group is unexposed. The number of samples for each group was 27. Data is analysed using chi-square and t-test. The result of the research has obtained the characteristic of the workers in the exposed group were the age of 20-30 years old, the working period of 3-5 years, 8 hours/day work, using personal protective equipment (PPE) and not smoking. The unexposed group have 20-30-year-old characteristics, 5-10 years of service, 8 hours/day work, no PPE and smoking. Macroscopic characteristics of semen liquefaction, viscosity, pH and colour of the two groups were no different. Semen volume under 1.5 ml of 18.5% (exposed) and 7.4% (unexposed) with  $p > 0.05$ ; OR=2.84. Microscopic characteristics of semen for motility and viability were not different in the two groups. Low spermatozoa number in exposure group was 55.6% and not exposed 18.5% with a  $p$  value  $< 0.05$  and OR 5.5. Normal spermatozoa morphology of exposed group workers was 28% and the group was not exposed to 34%. It could be concluded that there is a relationship between occupational exposure and semen analysis.

Keywords: semen analysis, fertiliser, factory workers

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### **1. Introduction**

Reproductive health problems arose more than a few decades due to exposure to chemicals [1]. Individually a person can be exposed to toxicants at home, school and workplace. Substances that potentially harm reproductive health can come from water, air, soil, dust and food. Some chemicals can have a negative effect on

male reproduction by killing and destroying cells that function in normal male reproduction or hormonal disorders. Both can lead to infertility in men. Toxicants can enter the body through inhalation, ingestion and absorption through the skin. Toxicant or its metabolites will go to target organs such as ovaries and testes that will give biological effects on the organ that can cause infertilities.

Plants need some essential elements other than oxygen, carbon, hydrogen that can be obtained from the soil. Essential elements are called nutrients. Macronutrients are needed in large quantities such as nitrogen, phosphorus and potassium. Fertilizers often contain these 3 elements. The source of nitrogen is ammonia ( $\text{NH}_3$ ), ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), sodium nitrate ( $\text{NaNO}_3$ ) and urea ( $\text{N}_2\text{H}_4\text{CO}$ ). Phosphorus is supplied from phosphates like  $(\text{NH}_4)_2\text{HPO}_4$ . Potassium obtained from potassium sulphate ( $\text{K}_2\text{SO}_4$ ) or potassium chloride ( $\text{KCl}$ ).

Urea fertilizer if dissolved with water will become ammonium bicarbonate. Nitrogen in the form of ammonium readily evaporates into the air as an ammonia gas. Urea is synthesized from ammonia and carbon dioxide [2]. Urea can also synthesize through ammonium carbamate which is dehydrated [3].

Research results exposure to environmental toxicity and work associated with poor sperm quality that is viability and the normal form of spermatozoa significantly reduced [4]. Other studies states that there is no relationship between contaminated water from deactivated fertilizer waste depository with reproductive changes (testicular weight, spermatozoa analysis, pregnancy rate and Sertoli cell count) in mice balb-c whereas in water from water treatment station there is a reduction of sex ratio from offspring [5]. Based on this description, a study of semen analysis as a means for evaluation of infertility status is important for couples in counselling their fertility choice.

## **2. Methods**

This type of research is a case-control with a case is the group exposed and the control is a group not exposed. The number of samples is 27 for each group. Semen preparation was taken by masturbation after the employee was asked to do abstinence or at least 2 days and no more than 7 days or the preparation must be delivered to the laboratory within one hour after discharge. The preparation is stored in a clean and wide-mouthed glass container and the container should be warmed first to reduce sharp temperature differences. The hand and penis should be washed before the preparation is accommodated. Human semen is examination and processing according to the laboratory manual [6,7].

Semen preparation from ejaculation in the form of semi-solid coagulated mass. These preparations will liquefaction within 15-60 minutes at room temperature. Preparations are immediately examined after liquefaction or within one hour after ejaculation. Normal liquid reaction semen samples will look homogeneous with grey-opalescent colour appearances, clear if the sperm quantity is too low, red-brown if there are red blood cells (haemospermia), yellow if taking vitamins or drugs. The volume of semen was measured using a graduated glass measuring cylinder with a wide mouth (0.1 ml accuracy). Lower reference limits 1.5 ml (95% CI 1.4-1.7).

The pH of semen is measured using pH paper in the range 6-10 should be used. The WHO manual retains the consensus value of 7.2 as a threshold value. Spermatozoa motility within semen should be assessed as soon as possible after liquefaction.

The semen sample is measured by their motility in a slide that is covered with a cover glass (22x22 mm) under a magnification microscope 200x. Assess approximately 200 spermatozoa for the percentage of different motile categories. The lower reference limit for total motility (Progressive + Non-Progressive) is 40% (95% CI 38-42). Progressive sperm motility is related to pregnancy rate.

Spermatozoa viability should be assessed as soon as possible after liquefaction of the semen sample. Spermatozoa viability was observed using eosin Y dye dripped on the tip of the object glass and then added one drop of semen (total 10 $\mu$ l), homogenized and made smear preparations. Viability observation was carried out on 200 cells under a light microscope with 400x magnification. Living spermatozoa will not be colour by eosin Y but those that have died will be purplish red due to damage to the cell plasma membrane. Tally the number of stained (dead) and unstained (live/vital) cells with the aid of laboratory counter. The lower reference limit for viability is 58% (95% CI 55-63).

The number of spermatozoa in the ejaculate is calculated from the concentration of spermatozoa, which is measured during semen evaluation. Sperm numbers are calculated using the Improved Neubauer haemocytometer. As much as 10  $\mu$ l of spermatozoa suspension from semen preparation was taken with a micropipette, then placed in a haemocytometer and then covered with a cover glass after which it was left for 10-15 minutes to be absorbed and settled in the area of calculation. Count only whole spermatozoa (with heads and tails) using the light microscope with 400x magnification. Lower reference limit form sperm

concentration is 15 x 10<sup>6</sup>/ml (9 5%CI 12-16 x 10<sup>6</sup>).

Morphology of spermatozoa was observed from smear preparations made on clean glass objects by dropping one suspension. Then the suspension was smeared with the help of another slide, the preparation of the smear was left to dry by itself. After dry, the preparation was fixed with 40% methanol for 5 minutes then rinsed with distilled water and dried. Then the object glass was dripped with Giemsa dye 3% and left for 30 minutes, then rinsed again with tap water and dried at room temperature. Observations were carried out with a 400x magnification microscope on 200 spermatozoa per treatment group. The lower reference limit for the normal form is 4% (95% CI 3-4). Data analysis was done by t-test for parametric data and chi-square for categorical data.

### **3. Result**

Characteristics of the study sample include age, years of service, length of work, use of personal protective equipment and smoking habits. The age of all samples from both exposed and unexposed groups is a minimum of 20 years and a maximum of 30 years old. This age is a productive age. The working period of all samples from both exposed and unexposed groups is a minimum of 3 years. The average duration of work for both exposed and unexposed groups is 8 hours per day. The use of complete personal protective equipment specified in groups exposed based on the results of the questionnaire while in the unexposed group did not use the special personal protective equipment. The exposed group all samples did not smoke based on the

results of the questionnaire while in the unexposed group most were smokers.

Semen parameters observed includes the macroscopic and microscopic examination. The macroscopic examination includes the liquefaction of the spermatozoa, normal if within 60 minutes it has liquid. The viscosity of the semen is examined immediately after the liquefaction,

normal if it is dripped from a pipette in the form of small droplets or does not form a thread more than 2 cm. The normal 7.2 as a lower threshold value; normal semen volume is 1.5 ml. Based on macroscopic data, semen exposed, and unexposed groups did not differ much. The results of the study are shown in table 1.

Table 1. Macroscopic characteristic.

No	Semen Macroscopic	Exposed (n=27)	Unexposed (n=27)
1	Liquefaction	normal	normal
2	Viscosity	normal	normal
3	Color	Grayish white	Grayish white
4	pH (mean±sem)	8,22±0,11	8,15±0,07
5	Semen volume (ml)	2,53±0,19	2,37±0,10

The microscopic examination includes the motility, viability, number and morphology of spermatozoa. The results of the microscopic examination are shown in table 2.

Table 2. Microscopic characteristics.

No	Spermatozoa Charateristic	Exposed (n = 27)	Not Exposure (n = 27)
1	P + NP motility (%)	77 ± 1.31	77 ± 1.24
2	Viability (%)	77 ± 1.86	77 ± 1.34
3	Number/concentration (million/ml) *	18 ± 1.88	53 ± 6.06
4	Normal morphology (%) *	28 ± 1.83	34 ± 1.69

\* (P <0.05), independent t-test

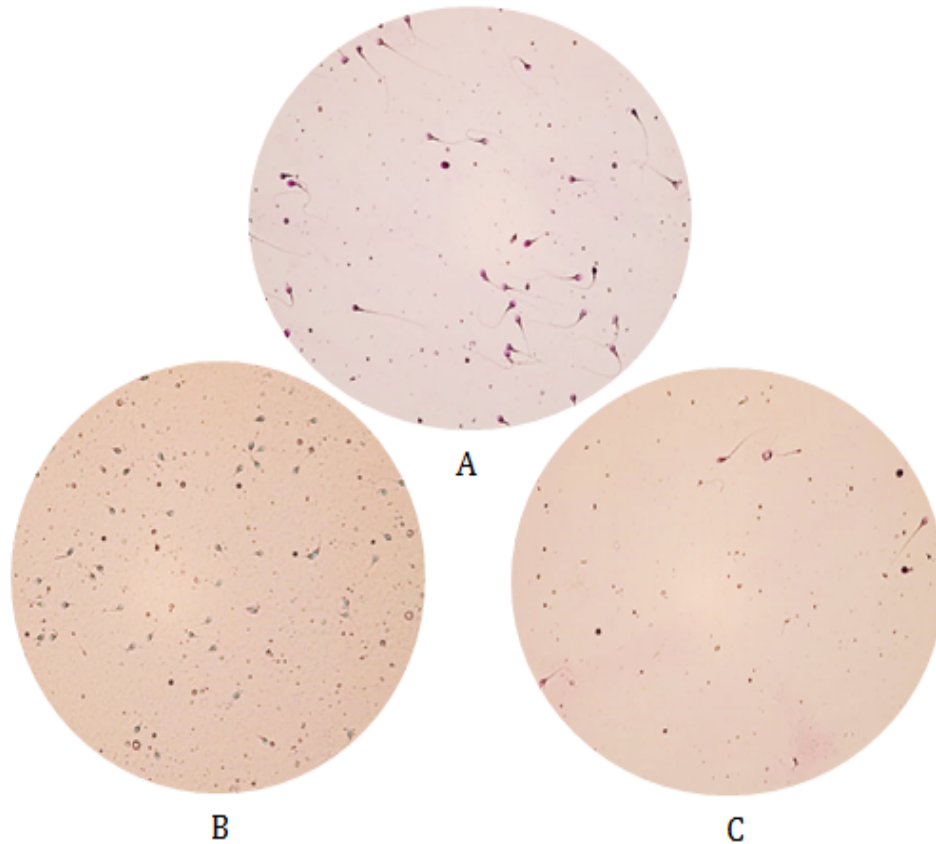
The microscopic data there is a difference in the number and morphology of the spermatozoa. In workers from the exposed group, there was the lowest number of spermatozoa 9 million/ml, this data was below the low limit of WHO which was 15 million/ml while the group workers were not exposed have the lowest value of 14 million/ml. the average value of

the two groups was significantly different from the results of the independent t-test where the number of spermatozoa exposed was significantly lower than the unexposed group.

Differences also occur significantly in the normal morphology of spermatozoa between group exposed and unexposed. Normal morphology in the exposed group is significantly lower

than the unexposed group. The group exposed to normal morphology was 28% and the group was not exposed 34%. The normal morphology of the group exposed to the minimum-

maximum value is 11-46% while not exposed to 15-49%.



**Figure 1.** The spermatozoa morphology (400 x magnifications) of the group control (A) and case (B & C).

The research data was tested by chi-square to analyze the relationship of

occupational exposure to the number of spermatozoa.

**Table 3.** Occupational exposures relationship with the number of spermatozoa.

Total (million/ml)	Case		Control		Total		p value	OR (95% CI)
	n	%	n	%	n	%		
Abnormal ( $<15$ )	15	55.6	5	18.5	20	37.0	0,011	5,500 (1.604-18.864)
Normal	12	44.4	22	81.5	34	63.0		
Total	27	100	27	100	54	100		

The normal number of spermatozoa in the group exposed to 12 people (44.4%), while abnormal as many as 15 people (55.6%). In the control group obtained a normal sperm count of greater value than the cases, accounting for 22 persons (81.5%) while the abnormal value is low, amounting to 5 people (18.5%). From the analysis

$\rho=0.011$  and  $OR=5,500$ . This means that there is a significant relationship between occupational exposure and the number of spermatozoa. It is characterized by values  $\rho=0.011<0.05$ . Then to analyse the relationship of occupational exposure to the volume of semen, chi-square test was performed, the results can be seen in table 4.

Table 4. Occupational exposures relationship with semen volume.

Volume (ml)	Case		Control		Total		p value	OR (95% CI)
	n	%	n	%	n	%		
Abnormal (<1.5)	5	18.5	2	7.4	7	13	0.420	2,841 (0.500 to 16.138)
Normal	22	81.5	25	92.6	47	87		
Total	27	100	27	100	54	100		

The normal volume of semen in the exposed group was as many as 22 people (81.5%) while the volume of less than 1.5 ml was 5 people (18.5%). In the unexposed group, the normal semen

volume was higher in value than the exposed group, which was 25 people (92.6%) while the abnormal (the volume of semen less than 1.5 ml) was lower at 2 people (7.4%).

#### 4. Discussion

Based on the macroscopic data of semen, the exposed and unexposed groups were not different, but the microscopic data of spermatozoa had a difference, namely the number and morphology of spermatozoa. In exposed group workers, there is the lowest number of spermatozoa 9 million/ml, this data is below the lower limit of WHO which is 15 million/ml while group workers are not exposed to the lowest value of 14 million/ml. Significant differences also occur in normal morphology between group workers exposed and not exposed. The

lowest value of the normal spermatozoa morphology of the exposed group is 11%. It's just that the lower limit of WHO in 2010 for normal spermatozoa morphology is 4% [6–8]. So that it can be said that the exposed group workers decreased the number of spermatozoa called oligozoospermia. Abnormalities that occur in spermatozoa may be caused by the work environment exposed to chemicals and high temperatures. Fertility in men can be reduced due to abnormalities or congenital abnormalities of the urogenital, urogenital tract infections, malignancies, endocrine disorders, genetics, immunology, environmental

influences; the increase is in the temperature of the scrotum and occupational exposure [9].

Several ways that spermatotoxicity is biologically acceptable are through the excess of reactive oxygen species and endocrine disrupting ingredients [10–12]. Reactive oxygen species are naturally produced by spermatozoa cells that are metabolically active, but excess reactive oxygen species results in damage to membrane and spermatozoa DNA which can further affect fertilization and embryogenetic integrity. Therefore, chemicals that because oxidative stress have the potential to become spermatotoxicant. Ammonia can induce production of free radical. Cigarette smoke, burning fuel oil, gases that contain reactive chemicals can cause oxidative stress. Endocrine disruptors can block androgen activity by binding to androgen receptors or be disrupting steroidogenesis so that testosterone production decreases. Workers associated with ammonia, excessive ammonia in the body affect glucose metabolism and insulin activity, the concentration of luteinizing hormone and steroid hormones decreases resulting in hormonal disorders.

Increased temperature directly inhibits spermatogenesis so that sperm production is disrupted. Increased scrotal temperature can cause decreased sperm quality and infertility. The temperature for optimum spermatogenesis is 2°C lower than the temperature in the body. Warming the scrotum in humans to 43°C for 30 minutes causes ejaculation sperm count to decrease by 80%. Spermatogenesis is temperature dependent. Testicular temperature is regulated by the scrotum and the thermal regulator system found on the spermatic cord. On the spermatic cord there is a heat exchange between

arterial blood and venous blood. Venous blood temperature is lower than arterial blood due to heat being lost through the skin in the scrotum. On the scrotum there is no subcutaneous fat and has a skin surface area that can cause changes in temperature. The damaging effects of heat results from a retrospective study of bakers and welds concluded that heat in the environment is a risk factor for male fertility. Men who wear tight underwear or are very close, riding a bicycle, taxi driver and sitting with a laptop on their laps, sitting at work 8 hour a day can increase the scrotum temperature so that the potential to increase testicular temperature is also [13–17]. Several studies state that heat temperature as risk factors and causes of infertility in men [18–20]. Fertilizer manufacturing process requires steam water, so it is a risk factor for their workers [21, 22].

## **5. Conclusion**

Based on the description of the results and discussion it can be concluded that there was a relationship of occupational exposure to semen analysis that is microscopic characteristic of the group exposed to oligozoospermia and morphology of normal spermatozoa in the exposed group was also significantly lower than the unexposed group. It is expected that workers understand the risks of work on their reproductive health.

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