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Reproductive Abnormalities in Men Due To Occupational Exposure.

Dereddy Naga Linga Reddy, Kadiri Pullanna, and Gundala Harold Philip*

Department of Zoology, Sri Krishnadevaraya University, Ananthapuram, Andhra Pradesh - 515003, India.

ABSTRACT

We made semen analysis of men occupationally exposed to pesticides and cement dust in order to establish whether exposures have any implications on reproductive health in male population of Anantapurm district, Andhra Pradesh, India. Twenty four (24) men of each occupation working in pesticide godown, cement godowns and involved in pesticide spraying were selected. Twelve (12) belonged to age group of 21-30 and twelve age group of 31-40. Semen parameters like volume, liquefaction, alkalinity, sperm count, motility and morphology were observed. Incidences of Hypospermia (51), Teratozoospermia (17), Asthenozoospermia (25), Oligozoospermia (57), Oligoasthenozoospermia (21), Oligoasthenoteratozoopermia (11) were observed in men of all three occupations indicating that these occupations are potential hazards for men.

Keywords: Occupational exposure, Men, Reproductive health, Semen analysis.

***Corresponding author**

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INTRODUCTION

Worldwide 10-15% of couples are affected by infertility which can be attributed to various factors. Male infertility plays a key role in conception difficulties of up to 40% infertile couples [1]. Approximately 50% of reported cases are accounted for by male factor infertility, resulting in a prevalence of about 7% of all men [2-4]. Low-wage populations for their lively hood disproportionately work in occupations associated with hazards workplace environment. Men especially to feed the family have no choice of occupation and hence there is a significant public health concern about the potential effects of occupational exposure to toxic substances on reproduction. Many toxicants with reported or suspected reproductive and developmental effects are still in regular commercial use and present potential exposure to workers.

Assessment of various reproductive health Indices of men with occupational exposure have been carried out around the globe. Alarming reports were there from 1880's with regard to decline in sperm count. Studies have shown that in some parts of the world, average sperm counts have dropped by 50% since the 1940 [5-6]. Reduced sperm count and sterility in workers manufacturing or applying the pesticide, dibromochloropropane was observed from 1970's [7]. Occupational exposure and environmental exposure to hazardous substances have been conjectured as one of the etiologies of sperm number decline [8]. Effects on male reproductive health due to various environmental contaminations have been reported earlier [8-9]. Recently Bonde and Giwercman [10] reviewed the relation between environmental xenobiotics and male reproductive health.

There has been a great development in epidemiological and laboratory methods to perform observational studies in humans; in particular with respect to functional measures of fertility and laboratory refinements of studies of semen quality [11-12]. Most research on human male reproductive health has been stimulated by studies of the effects of exposures in animals and their offspring, surveillance and anecdotal observations also have led to investigations of male reproductive exposures [13].

In this investigation we analysed the consequences of occupational exposures on reproductive health of males in Anantapur district, Andhra Pradesh by examining certain key parameters related to male reproduction. The study populations consisted of men working in pesticide godowns, pesticide spraying and cement factories for more than from five (5) years. Semen analysis for years has remained the benchmark measurement of male fertility. Hence this study would be of great utility as adverse health effects observed in case studies provide clues to potential health effects that should be studied to determine no effect levels.

MATERIALS AND METHODS

Two hundred healthy human males who are occupationally involved in spraying of pesticides, working in pesticide godowns and working in cement godowns were interviewed particularly regarding age and time of exposure. From this sample seventy two (72) persons were selected and divided into three groups. Each group consisted of twenty four (24) men who are involved in these three occupations. Out of this twenty four, twelve are of the age group of 21-30 and twelve in the age group of 31-40 years. The study protocol was approved by the Institutional Animal Ethical Committee. Before enrollment in the study, written consent was obtained from volunteers.

The selected men were invited to clinical laboratory and semen sample was collected by masturbation and ejaculated into a clean wide mouth glass container. Care was taken to see that the sample was collected after a minimum of two days and maximum of seven days sexual abstinence. The semen sample collected was kept at room temperature (20°C-37°C) to avoid any effect on spermatozoa. Container was labeled with person's name, identification number, date and time of collection. WHO guidelines were followed in collection and analysis of semen sample. [14] The following investigations were carried out in the samples.

Colour, volume and pH

Colour of the semen was observed immediately after collection and the volume was measured using graduated test tube. The semen reaction was observed by measuring its pH.

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Liquefaction

Immediately after ejaculation into the collection vessel sample was kept at room temperature and time of liquefaction was observed to 90 min. Semen was typically a semisolid coagulated mass first and within a few minutes at room temperature, the semen usually begins to liquefy (become thinner). The time taken to liquefy was noted.

Sperm count and motility

Sperm count and motility were made using the above liquefied sample under the microscope. Total sperm count (Mill/ml) was calculated by using neubauer chamber [14]. Briefly the liquefied semen was diluted 1:20 with sodium carbonate and this diluted sample was placed on the neubauer chamber and counted under the microscope (Labomed). Motility was determined by counting the number of motile and immotile spermatozoa from the same slide in several randomly selected fields under 20X objective until at least 200 spermatozoa were counted. The minimum of five microscopic fields were examined.

Sperm morphology

This was determined with the help of smears made from semen samples using feathering technique. A clean glass slide was taken, washed in 70% ethanol and dried. A small drop of semen (5 to 20 µl) was taken onto the slide. The edge of a second slide was placed on the first, at an angle of 45° and the semen drop was dragged along the surface to make a thin smear. These were then air dried and fixed. Sperm morphology was evaluated using hematoxylin and eosin stain. Normal and the abnormal sperms were observed under 100X oil immersion microscope. Each of the spermatozoa was examined for head, mid–piece and tail defects. A total of 200 spermatozoa were observed for defects and expressed in percentage. Loose heads were counted (as abnormal forms), while free tails were not counted. Structures without any head anterior to the basal plate were not counted.

RESULTS

Data on semen analysis of men who were occupationally exposed to either pesticides or cement dust are given in tables 1 to 4. In this investigation we found that all men had alkaline semen. The volume of semen measured was less than the normal values in fifty one (51) men out of seventy two (72). In men whose occupation was pesticide spraying eight persons in the age group of 21-30 and nine persons in the age group of 31-40 have shown hypospermia (Table 1). In men who work in pesticide godowns, nine persons in the age group of 21-30 and eight persons in the age group of 31-40 have shown hypospermia (Table 2). In men whose occupation was work in cement factory eight persons in the age group of 21-30 and nine persons in the age group of 31-40 have shown hypospermia (Table 3).

Table 1: Semen analysis of men involved in spraying of pesticides.

S.No	Parameters Examined	Normal Values	Age:21-30	Age:31-40
1	Colour	Grey-opalescent	Grey-opalescent	Grey-opalescent
2	Reaction	Alkaline	Alkaline	Alkaline
3	Volume	1.5-5ml	1.45±0.74 (0.5-2.0)	1.35±0.51 (1.0-2.5)
4	Liquefaction	15-60mins	31.66±21.92 (15-90)	36.66±21.34 (15-90)
5	Sperm count	39-150mill/ml	24.58±15.01 (9-57)	27.75±18.06 (9-65)
6	Total motility	32%	48.33±13.05 (30-70)	47.58±18.35 (0-70)
7	Morphology	4%	3.91±0.27 (3-4)	3.41±1.11 (3-4)

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Table 2: Semen analysis of men working in pesticide godown.

S.No	Parameters Examined	Normal Values	Age:21-30	Age:31-40
1	Colour	Grey-opalescent	Grey-opalescent	Grey-opalescent
2	Reaction	Alkaline	Alkaline	Alkaline
3	Volume	1.5-5ml	1.24±0.53 (0.5-2.5)	1.48±0.59 (1.0-2.5)
4	Liquefaction	15-60mins	33.75±17.57 (15-75)	37.50±24.10 (20-90)
5	Sperm count	39-150mill/ml	26.00±12.58 (15-50)	25.75±16.44 (9-72)
6	Total motility	32%	51.00±11.97 (35-70)	41.58±7.98 (35-60)
7	Morphology	4%	3.83±0.37 (3-4)	3.91±0.75 (3-6)

Table 3: Semen analysis of men working in cement godown.

S.No	Parameters Examined	Normal Values	Age:21-30	Age:31-40
1	Colour	Grey-opalescent	Grey-opalescent	Grey-opalescent
2	Reaction	Alkaline	Alkaline	Alkaline
3	Volume	1.5-5ml	1.36±0.60 (0.5-2.5)	1.35±0.51 (1.0-2.5)
4	Liquefaction	15-60mins	32.50±22.59 (15-90)	40.41±25.69 (20-90)
5	Sperm count	39-150mill/ml	22.66±14.30 (9-57)	31.75±18.22 (9-72)
6	Total motility	32%	49.33±11.50 (35-70)	44.58±12.81 (35-70)
7	Morphology	4%	3.66±0.47 (3-4)	3.58±1.18 (3-5)

Table 4: Sperm Analysis of male persons in the age group of 21-30 and 31-40 years exposed to pesticides and cement as a result of their involvement in spraying, godown worker and cement factory workers.

S.No	Occupational exposure	Age group in years	Abnormalities in number of men
1	Spraying (Pesticide)	21-30	Oligozoospermia-10, Hypospermia-8, Oligoasthenozoospermia-4, Asthenozoospermia-5 Teratozoospermia-2, Oligoasthenoteratozoopermia-2.
		31-40	Hypospermia-9, Oligozoopermia-9, Asthenozoospermia-3, Teratozoospermia-4, Oligoasthenozoospermia-2, Oligoasthenoteratozoopermia-2.
2	Godown worker (Pesticide)	21-30	Oligozoospermia-9, Hypospermia-9, Teratozoospermia-2, Oligoasthenozoospermia-2, Asthenozoospermia-2, Oligoasthenoteratozoopermia-1.
		31-40	Hypospermia-8, Oligoasthenozoopermia-5, Teratozoospermia-3, Oligozoospermia-11, Asthenozoospermia-6, Oligoasthenoteratozoopermia-3.
3	Cement dust (cement factory)	21-30	Oligozoospermia-10, Hypospermia-8, Teratozoospermia-4, Oligoasthenozoospermia-3, Asthenozoospermia-3, Oligoasthenoteratozoopermia-1.
		31-40	Hypospermia-9, Oligoasthenoteratozoopermia-2, TeratozooSpermia-2, Oligozoospermia-8, Asthenozoospermia-6, Oligoasthenozoospermia-5.

Liquefaction time of semen observed in men involved in all three occupations was within the time given by WHO in all age groups (Table 1-3). With regard to sperm count it was less than the normal values in

fifty seven (57) men out of seventy two (72) men who were examined. In men whose occupation was pesticide spraying ten persons in the age group of 21-30 and nine persons in the age group of 31-40 have shown oligozoospermia (Table 1). In men who work in pesticide godowns, nine persons in the age group of 21-30 and eleven persons in the age group of 31-40 have shown oligozoospermia (Table 2). In men whose occupation was work in cement factory ten persons in the age group of 21-30 and eight persons in the age group of 31-40 have shown oligozoospermia (Table 3).

In this study sperm motility were less than the normal values in twenty five (25) men out of seventy two (72) in men whose occupation was pesticide spraying. five persons in the age group of 21-30 and three persons in the age group of 31-40 have shown asthenozoospermia (Table 1). In men who work in pesticide godowns, two people in the age group of 21-30 and six people in the age group of 31-40 have shown asthenozoospermia (Table 2). In men whose occupation was work in cement factory three persons in the age group of 21-30 and six persons in the age group of 31-40 have shown asthenozoospermia (Table 3).

Morphologically abnormal sperms have been noticed in fifteen men (17) out of seventy two (72) men examined. In men whose occupation was pesticide spraying two persons have shown Teratozoospermia in the age group of 21-30 and four persons in the age group of 31-40 have shown Teratozoospermia (Table 1). In men who work in pesticide godowns two persons have shown Teratozoospermia in the age group of 21-30 and three persons in the age group of 31-40 have shown Teratozoospermia (Table 2). In men whose occupation was work in cement factory four persons have shown Teratozoospermia in the age group of 21-30 and two persons in the age group of 31-40 have shown Teratozoospermia (Table 3).

DISCUSSION

Infertility is a reproductive health problem that affects many couples in the human population. The issue of male infertility caused by occupational exposure is pertinent worldwide as it has been inevitable for man to live in an environment which has several toxic compounds. Relationship between occupation background and semen impairment have been well established. This study investigated this relationship using a questionnaire especially to know about their occupation. The three occupational exposures identified are independent of each other.

Semen which has a very high buffering capacity than most other fluids in the body was observed for its colour abnormality. It was good to notice that there was no change in the color of the semen which was collected from all seventy two men who were involved in three different occupations. WHO considers 1.5 ml as the lower reference limit with regard to semen volume [15]. But in clinical practice, men ejaculating a volume of less than 2ml are considered infertile. In this study fifty (50) men out of seventy two (72) have shown lower semen volume, a condition known as hypospermia, indicating that occupations at these three work places had adverse effect on semen volume. Though this is relatively common issue in men of any age, low testosterone level and nutritional deficiencies could be the factors in the influencing. Psychogenic anorgasmia may also be the reason for low-volum [16]. Our results are in confirmity with the observations made in men involved of different occupations by other researchers [17-18]. Vaginal cervical region of the female reproductive tract are acidic, so the alkalinity of the semen neutralizes the same and prevents destruction of spermatozoa in it. We did not notice any abnormal change in pH even in one person.

Semen or seminal fluid is an organic fluid that contains spermatozoa. In humans, seminal fluid contains several components like proteolytic enzymes, citric acid, acid phosphatase, lipids and fructose besides spermatozoa [19]. Sperm makes only 1 to 10% of the semen. Semen is a thick gel at the time of ejaculation and normally becomes liquid with in 20minutes (or 15 to 60mins) after ejaculation. The thick gel is formed by proteins from the seminal vesicles. The liquefaction time is a measure of time it takes semen to liquefy. Tauber et al [20] have shown that liquefaction occurs only in a pH range of 6.8-8.8, at which pepsin is not active. If liquefaction is delayed it will be difficult for sperm to break thick semen. Hence the semen must liquefy quickly to allow sperm to swim out of acidity of the vagina. We did not notice liquefaction to be on issue in men of these three occupations.

Reduced sperm count was observed in fifty seven (57) out of seventy two (72) subjects examined, which is quite alarming. The present findings are consisted with previous studies which demonstrated decreased sperm count under various situations [21-23]. Sperm motility has been shown, in several studies, to

be a good predictor of human male fertility *in vivo* and in vitro [24]. Sperm motility has also been found to be strongly associated with the probability of conception [25-26]. As the sperm cells are motile, their motility in percent was used to grade the quality of semen. Zhang et al [27], reported that reduced epididymal sperm count and motility and testosterone levels in mice exposed to permethrin. It was shown that fenvalrate and cypermethrin impact sperm motility and sperm count directly or indirectly by affecting spermatogenesis via interaction with androgens or their receptors in rats [28]. Sperm motility was significantly decreased with the highest concentration of organophosphate pesticides (i.e., Chlorpyrifos methyl, diazinon and profenofos) in male rats [29]. Reduced motility in human subjects was also reported [30-31, 21].

Normal sperm have an oval head and long tail. Abnormality could be defective heads**/**tails. If semen sample contains 4% of morphologically normal forms, it is confidential fit. Observations made in this study are also supported by investigation carried out by others. Ten et al [32] have shown declining semen quality in humans due to environmental pollutants, occupational exposures or changes in lifestyle. Kamijima et al [33] observed deterioration of sperm motility and morphological changes in men involved in spraying of insecticide. Meltem et al [34] observed Methyl parathion induced significant decrease in sperm counts and sperm motility, increase in abnormal sperm morphology.

CONCLUSION

Our observations of semen quality in men occupationally exposed to pesticides and cement dust have shown their advance effects on sperm count, volume, motility, liquefaction and morphology. Deterioration in semen quality appears in direct proportion to the occupational exposure.

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