BioMetals **12:** 339–346, 1999.

Male reproductive effect of nickel sulphate in mice

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Received 16 June 1999; accepted 4 August 1999

Key words: nickel sulphate, enzymes, mice, sperm testis, epididymis, seminal vesicle

Abstract

Nickel sulphate was administered orally to adult male mice at dose level of 5 and 10 mg/kg body weight (5 days per week) for 35 days. There was no change in body weight. However a significant decrease in absolute and organ-to-body weight ratios of testes, epididymides, seminal vesicles and prostate gland was observed. The sperm abnormality, associated with decrease in sperm motility and sperm count was also observed. Significant alterations in the activities of marker testicular enzymes, viz. sorbitol dehydrogenase (decreases), lactate dehydrogenase (increases) and γ-glutamyl transpeptidase (increases) associated with histopathological changes in testes, epididymides and seminal vesicles, were also observed. Accumulation of nickel in testes, epididymides and seminal vesicles was also observed. The study reveals that the oral exposure to nickel may affect the histology of testes, epididymides, seminal vesicles and sperms morphology. These testicular and spermatotoxic changes may be responsible for observed male mediated developmental toxic effects.

Introduction

The industrial application of nickel has very broad spectrum, primarily it is used in alloys. The other uses are in electroplating, welding, flame cutting, flame spraying and mold making. Nickel is also used in the manufacture of jewellery, coinage, cutlery, cooking utensils and dental or surgical prostheses. Nickel has been reported to leach out from the utensils used during cooking and storage of foods (EHC 1991). Human exposure to nickel may occur in industrial environment or through food chain. Nickel is absorbed through oral, dermal and inhalation routes as well (Toxicology Profile for Nickel 1997).

The dietary requirement of nickel is 50–80 ng/gm of diet (Prasad 1976). Higher quantity of nickel is known to be injurious for human health. It creates allergy, cancer, non malignant respiratory tract disorders and iatrogenic nickel poisoning. Nickel hypersensitivity also causes asthma, conjunctivitis, inflammatory reactions. In acute exposure neurological symptoms, adrenal insufficiency, hyperglycemia, hepatic toxicity, renal damage, nasal and lung cancer may also develop. Nickel crosses the placental barrier effecting directly the developing embryo/foetus in experimental animals (Toxicology Profile for Nickel 1997).

Exposure to mice and rats, through inhalation route, is known to cause the testicular damage (Benson *et al.* 1988). In view of the exposure to nickel through food chain and paucity of information on its effects on male reproductive system, in the present investigation, the studies have been under taken to evaluate the effect of orally administered nickel on histoarchetecture of different compartments of the testes, accessory sex organs (epididymis, seminal vesicles), on sperm count, sperm motility and abnormalities in different regions of spermatozoa. The study also describes the bioaccumulation pattern of nickel in reproductive organs, effects on enzymes considered to be marker of testicular function and male mediated developmental toxic effects.

Materials and methods

The nickel sulphate of AR grade was procured from E-Merck. All other chemicals used in study were of the highest purity available.

Animal treatment

Adult male albino mice of swiss strain, body weight 25 ± 5 g, obtained from animal breeding colony of the Industrial Toxicology Research Centre, Lucknow, were used. The animals were fed on pellet diet (Lipton India, Limited) and water ad libitum, and maintained under standard laboratory conditions. Sixty mice were divided into three groups of twenty animals each. The animals of groups II and III recieved 5 and 10 mg nickel sulphate/kg body weight/day by gavage five days per week, for thirty five days. The desired amount of nickel sulphate was dissolved in distilled water and 0.2 ml solution was administered by gavage. The mice in group I received an equal volume of distilled water and served as vehicle control. The body weight of mice were recorded at the time of initiation and completion of the experiment. Prior to the termination of the experiment, mice were fasted over night, weighed and sacrificed by cervical dislocation on 36th day of experiment. Testis, epididymis, prostate gland and seminal vesicle were quickly removed and weighed.

The testes,epididymides and seminal vesicles of ten mice from each group were used for determination of nickel contents. The tissues from remaining ten mice of each group were used for histopathological and biochemical studies.

Spermatozoa count

Epididymal sperms were obtained by mincing epididymis in normal saline and filtering through nylon mesh. The sperm were counted using Neubauer Chamber (Freund & Carol 1964).

Sperm motility assay

The motility of sperm was assayed microscopically within 5 min following their isolation from cauda epididymis at 37 °C and data were expressed as percent motility (Adelman & Cahill 1936).

Morphological abnormalities

The morphological abnormalities in sperm were enumerated by the methodology as reported by Hemavathi & Rahiman (1993) using light microscope.

Testicular enzyme assay

A portion of testis was homogenised (1:9) in 0.2 M Tris/HCl buffer pH 7.0 having 0.1% cetyltrimethylammonium bromide (CTNB) using Potter Elvejham homogeniser for the estimation of sorbitol dehydrogenase (SDH) following the method of Gerlach (1983). In the same homogenate lactate dehydrogenase (LDH) was also estimated (Vasault 1983).

Another portion of testis was homogenised (1:9) in 0.05 M Tris/HCl buffer pH 7.4 for the assay of γ-glutamyl transpeptidase following the methodology of Roomi & Goldberg (1981). Protein content of the sample were estimated by the method of Lowry *et al.* (1951).

Histological techniques

Testes and other accessory sex organs were fixed in 10% neutral buffered formalin, dehydrated and embedded in paraffin. Sections from each block (5 μm) were prepared and stained with haematoxylin-eosin following the standard procedures.

Nickel analysis

The testis,epididymis and seminal vesicle were soaked on the filter paper and weighed immediately. The tissues were digested twice with the nitric acid and finally with acid mixture of nitric acid, perchloric acid and sulphuric acid ($2 + 1 + 0.5$ ml). The digested samples were dissolved in 1% HNO_3 and made up to 5 ml. In the similar fashion an acid blank was prepared. The processed and digested samples were analysed on Atomic Absorption Spectrophotometer (Perkin Elmer model-5000) using air acetylene flame.

Male mediated developmental toxicity studies

Twenty male mice of proven fertility were orally administered 10 mg nickel sulphate/kg body weight 5 days in a week for a period of 35 days. Similarly twenty male mice were given equivalent amount of distilled water in an identical manner which served as vehicle control. The treated male and non treated female of proven fertility were housed overnight on a 1:3 basis in the home cage of the male. The maximum duration of pairing was 1 or 2 weeks. Positive evidence of copulation was confirmed by the presence of sperm in vaginal smear taken each morning during cohabitation (Dunnick *et al.*, 1984). The day on which evidence of copulation identified, was termed day zero

Table 1. Effect of nickel sulphate exposure on organ weights of mice

Group		Testis	Epididymis	Accessory sex organs	
				Seminal vesicle	Prostate gland
I ($n = 10$)	A	0.199±0.077	0.056±0.008	0.175±0.009	0.035±0.012
	B	0.787±0.158	0.266±0.054	0.694±0.165	0.135±0.001
II ($n = 10$)	A	0.165±0.021*	0.045±0.005	0.173±0.041	0.021±0.025
	B	0.676±0.236*	0.218±0.075	0.485±0.095*	0.101±0.001*
III ($n = 10$)	A	0.153±0.023*	0.048±0.005	0.223±0.012*	0.018±0.006*
	B	0.625±0.262*	0.191±0.034*	0.328±0.232*	0.072±0.005*

* $p < 0.05$.
A–Absolute organ weight (g).
B–Relative organ-to-body weight (g).
Mean ± S.E. of requisite number of mice in each group.
Group I – Control.
Group II – treated with 5 mg nickel sulphate / kg body weight.
Group III – treated with 10 mg nickel sulphate / kg body weight.

of gestation. The number of pregnant mice of nickel sulphate exposed or the control group were recorded for determination of fertility index. On the 18th day of gestation laparotomies were performed, number of corpora lutea were counted and foetuses were removed by uterine section. The number of live and resorbed foetuses (embryo) and total number of implantations were recorded. Fertility index, preimplantation loss and post implantation loss were calculated. Foetal weight and crown rump length were also recorded.

Statistical analysis

The data were statistically analysed using Student's *t*-test (Fisher 1950). $p < 0.05$ was considered significant.

Results

General toxicity

The animals at 5 and 10 mg/kg body weight dose did not show any mortality. The animals exposed to 10 mg/kg body weight, showed alopecia and sluggishness.

Body gain profile

The nickel sulphate treatment lead no effect on the body weight gain profile of animals (data not shown).

Organs weight

There was decrease in absolute and relative weight of testis, epididymis, seminal vesicle and prostate gland (Table 1).

Spermatology

The effect of nickel sulphate exposure on total epididymal sperm count and sperm motility is shown in Table 2. The results indicate decrease in total epididymal sperm count and in percent sperm motility. The data presented in Table 3 indicate a significant increase in morphological abnormalities in different regions of spermatozoa of mice exposed to 5 and 10 mg nickel sulphate/kg body weight.

Abnormalities were observed in head, neck & tail of spermatozoa. Abnormality include rephrase, anvil shaped, detached or enucleated head. Neck and tail

Table 2. Effect of nickel sulphate exposure on sperm motility and total epididymal sperm count

Group		Sperm motility (percent)	Total sperm count (per epididymis)$\times 10^7$
I	($n = 10$)	82.5±2.88	9.30±1.97
II	($n = 10$)	80.5±3.90	8.11±2.14
III	($n = 10$)	76.75±3.94*	5.87±1.18*

Mean ± S.E.
* $p < 0.05$.
Group I – Control.
Group II – Treated 5 with mg nickel sulphate / kg body weight.
Group III – Treated with 10 mg nickel sulphate / kg body weight.

may be coiled zig-zag or may be present in the form of slight impressions.

Enzymatic studies

Nickel Sulphate treatment on the specific activities of marker testicular enzymes lactate dehydrogenase, sorbitol dehydrogenase and γ-glutamyl transpeptidase is shown in Table 4. The results indicate significant decrease in the activity of sorbitol dehydrogenase and an increase in the activity of γ-glutamyl transpeptidase and lactate dehydrogenase at 5 and 10 mg/kg body weight doses of nickel sulphate.

Histopathology

In the normal testes seminiferous tubules are compactly arranged with well developed germinal epithelium as well as sperm cells in each tubule (Figure 1A). The histopathological examination of testes obtained from mice treated with 5 mg/kg body weight of nickel sulphate showed almost normal structural appearence of seminiferous tubules with succesive stages of transformation of germinal cells and Leydig cells. However, 10 mg/kg dose of nickel sulphate caused moderate congestion in the peripheral region, most of the seminiferous tubules located centrally appeared atrophied with increase in intertubular spaces, and there was evidence of disturbed spermatogenesis (Figure 1B).

The caput epididymides did not show any change in histoarticulature but cauda epididymides showed degeneration of epithelial cells, sperm were either absent or few in number. In the cauda epididymides of control mice the short columnar epithelium consisted of principal cells with elongated nucleus (Figure 2A). In Nickel treated mice, though the epithelium appeared regressed, the principal cells became vacuolated. Several principal cells lacked nuclei. Large spherical granules of varying sizes were abundant in the cytoplasm (Figure 2B). In the control mice, such granules were present in the perinuclear area of the principal cells, were smaller and fewer than those of nickel treated mice.

The seminal vesicles are made up of vesicular epithelium. Nickel sulphate treated animals showed reduction in the size of vesicles (Figure 3B). The seminal vesicle of control mice consisted of secretory vesicular epithelium. The vesicular folds were large (Figure 3A).

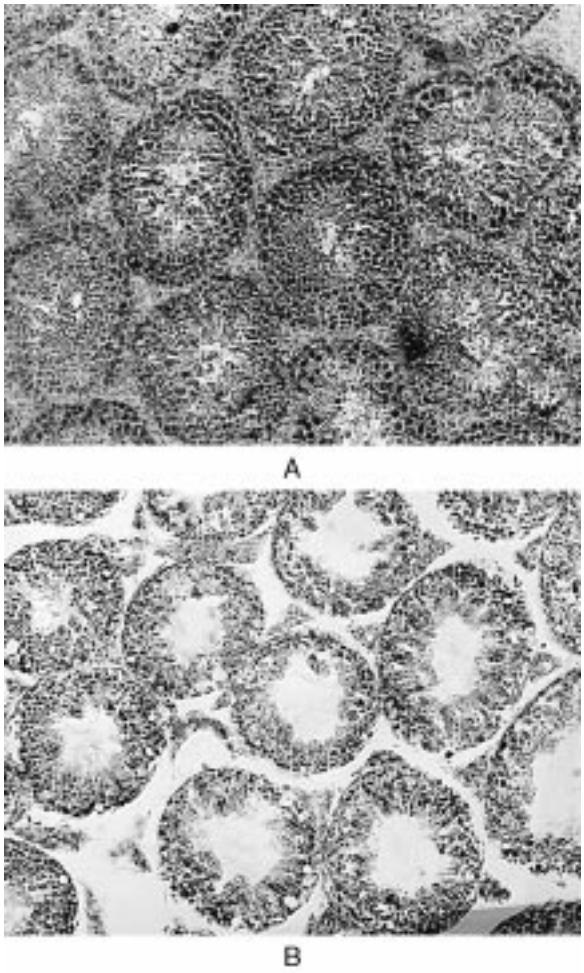

Figure 1. (A) Control testis shows compact seminiferous tubules, tubules have well developed germinal epithelial cells (HE&160). (B) In treated mice at 10 mg/kg b.wt. intertubular space and loss in spermatids (HE&160).

Accumulation of nickel

Nickel was present in appreciable quantity in the male reproductive organs even in control animals and it is clear that after the oral administraion of nickel sulphate at dose of 5 and 10 mg/kg body weight resulted in significant amount of nickel accumulation in testis, epididymis, seminal vesicle and prostate gland (Table 5).

The degree of accumulation of nickel in the male reproductive organs of treated animals was observed as follows:

Epididymis > Testis > Seminal vesicle > Prostate gland.

Table 3. Effect of nickel sulphate exposure on different types of morphological abnormalities in mice sperm

Group	Percent Abnormalities								Percent total
	Head	Neck		Tail					
	Detached	Curved	Bent	Curved	Bent	Round	Loop	Signet	
I ($n = 10$)	2.14±0.17	2.20±0.45	0.05±0.02	1.90±0.04	1.04±0.38	0.77±0.11	0.315±0.005	0.302±0.006	8.7±0.59
II ($n = 10$)	4.78±0.24	4.19±0.28	0.90±0.54	4.21±0.23	1.73±0.10	1.17±0.18	1.00±0.07	0.91±0.07	18.9±1.49*
III ($n = 10$)	5.68±0.13	4.96±0.14	3.09±1.93	5.54±0.19	1.46±0.38	1.38±0.03	1.39±0.30	1.62±0.44	25.14±2.41*

* $p < 0.05$.
Group I – Control.
Group II – Treated 5 with mg nickel sulphate / kg body weight.
Group III – Treated with 10 mg nickel sulphate / kg body weight.

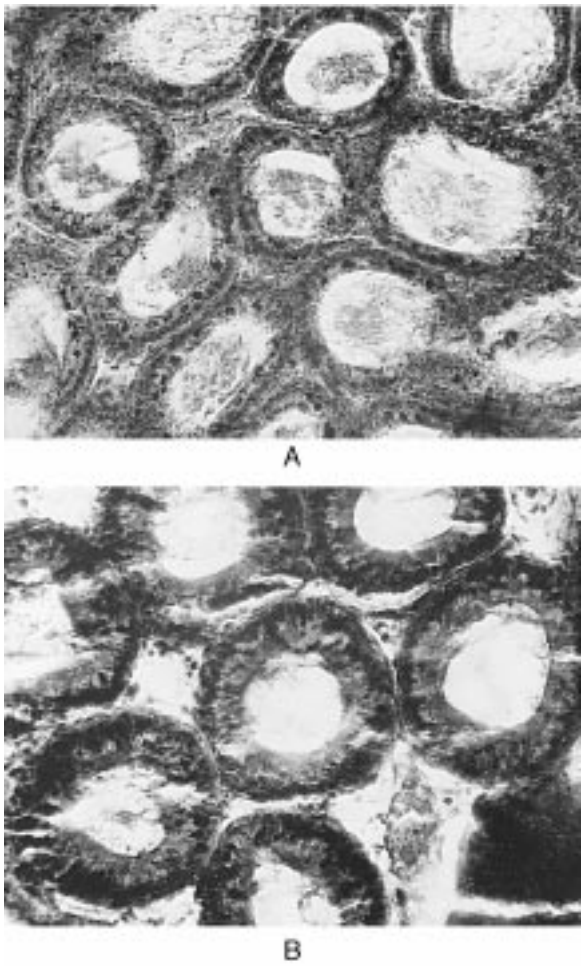

Figure 2. (A) Control cauda epididymis shows well develop columnar epithelium with few granules in cytoplasm of epithelial cells (HE&160). (B) In treated mice at 10 mg/kg b.wt.loss of columnar epithelium and presence of large sized granules in cytoplasm of epithelial cells (HE&160).

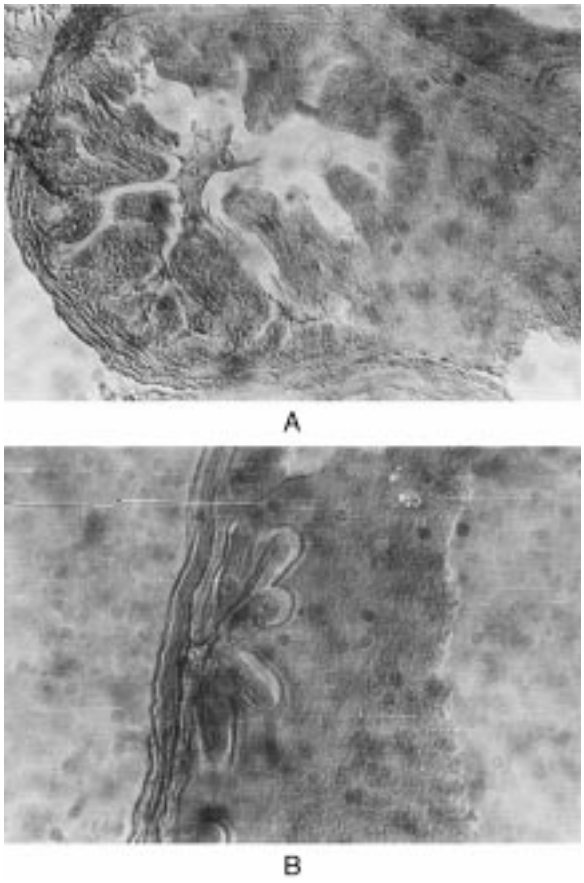

Figure 3. (A) Control seminal vesicle shows well developed vesicular epithelium (HE&160). (B) In treated mice at 10 mg/kg b.wt. loss in size of vesicular folds (HE&160).

Male mediated developmental toxicity

No treatment related mortality was observed in the mice during study period. The fertility index of exposed male mice were calculated on their ability to impregnate unexposed female mice. At 10 mg/kg body

Table 4. Effect of nickel sulphate exposure on marker testicular enzymes in mice

	Group I ($n = 10$)	Group II ($n = 10$)	Group III ($n = 10$)
γ-glutamyl transpeptidase	28.76±2.16	35.23± 4.50	38.44±2.24*
Sorbitol dehydrogenase	7.88±0.78	6.00±0.86	4.01±0.86*
Lactate dehydrogenase	194.22±3.15	236.7 ±0.023	243.86±0.017*

Enzyme activities are expressed as specific activities (n moles substrate oxidised or product formed/min/mg protein).
* $p < 0.05$.
Group I – Control.
Group II – Treated with 5 mg nickel sulphate / kg body weight.
Group III – Treated with 10 mg nickel sulphate / kg body weight.

weight dose level, fertility index was 46.6% in comparision to control 66.6%. The number of corpora lutea were almost similar. The number of implantation and resorption were 12.40±0.82 and 10.93±1.16 and 0.57±0.05 and 1.80±0.77 respectively in control and treated group. A significant percent in pre and post implantation loss was observed in treated group. There was no effect on crown rump length however foetal weight of dams mated with nickel treated males were decreased.

Discussion

There was no change in body weight of exposed animals, however a significant decrease in weight of testis, epididymis, prostate gland and seminal vesicle occured at dose of 10 mg/kg body wight. The decrease in weight of testis may be due to degeneration of germinal epithelium. The observed loss in weight of epididymis, and the seminal vesicle may be due to decrease in sperm count and morphological changes in these organs. The decrease in weight of testis and weight of accessory sex organs have been reported in experimental animal following exposure to the chemicals such as carbofuran (Pant *et al.* 1995), sodium selenite (Nebbia *et al.* 1987) and lead (Ronis & Badger 1996) which are known to exert testicular and spermatotoxic effects. An increase in abnormalities was observed in different regions (head, neck and tail) of spermatozoa. The abnormalities were found at 5 and 10 mg/kg body weight dose. Earlier investigators detected abnormalities only in the head region of the spermatozoa at the 28 mg/kg body weight dose of nickel sulphate (Sobti & Gill 1989).

The studies have shown that the activity of certain enzymes are associated with specific cell type of testis of germ cell maturation (Blackshaw 1970; Hodgen & Sherins 1973; Sherins & Hodgen 1976). The decreased activity of testicular enzymes viz. sorbitol dehydrogenase which is known to be associated with germ cell maturation along with increased activity of γ-glutamyl transpeptidase and lactate dehyrogenase (the enzymes related with the Sertoli cells and germinal epithelium respectively) indicate effects on these testis cell types from different doses of nickel sulphate. The histopathological changes observed in germinal epithelium of exposed animals, further support our observation. The alteration in the activities of marker testicular enzymes associated with the histopathological changes may be responsible for production of decreased number of spermatozoa. Nickel may cause various testicular damage via intratesticular and subcutaneous exposure to nickel nitrate (Kamboj & Kar 1964) and other testicular changes due to long term oral administration of nickel sulphate (Waltschewa 1972). Similar changes have been reported following exposure to phthalate esters, acrylonitrile, methylmethycrylate, styrene (Srivastava *et al.* 1992), hexachlorocyclohexane, carbaryl and carbofuran (Pant *et al.* 1995). It is well doccumented that nickel passes through the blood testis barrier (Dixon 1986). Such an accumulation of nickel in testis may be causing degeneration of germ cells as observed following methylmercury, mercuric chloride, molybdenum, boron, arsenic and lead poisoning in experimental animals (Dixon 1986). In the caput epididymis the sperm remain pack up since they are non motile acquire motility and complete maturation in cauda epididymis. Therefore spermatozoa in epididymis of this region absorb nickel very quickly (Hoey 1966) and adversely effects the histology. The observed changes in epithelial lining of cauda epididymis could interfere in acquiring motility of sperm. This may be one of the factor responsible for observed increase in non motile and abnormal sperm, the heavy metal nickel may exert deleterious effect on cell viability (Ng 1990). This is well in accordance with the toxicity reported for mercuric chloride (Sharma *et al.* 1996).

Seminal vesicle secretes fructose, proteins, enzymes phosphorylcholine and prostaglandins for the maintenance of the spermatozoa. The observed histopathological changes in secretory folds of epithelium and reduction in their size suggest the secretory

Table 5. Accumulation of nickel in different reproductive organs

Group	Testis (μg/g)	Epididymis (μg/g)	Seminal vesicle (μg/g)	Prostate gland (μg/g)
I ($n = 10$)	1.65±0.30	1.47±0.19	1.68±0.37	1.48±0.44
II ($n = 10$)	12.41±1.22*	14.95±0.86*	7.01±1.2*	1.65±0.48
III ($n = 10$)	16.18±2.44*	17.21±1.40*	11.86±1.28*	1.73±0.54

Mean ± S.E.
* $p < 0.05$.
Group I – Control.
Group II – Treated with 5 mg nickel sulphate / kg body weight.
Group III – Treated with 10 mg nickel sulphate/kg body weight.

Table 6. Male mediated embryotoxicity studies with nickel sulphate

	Control	Nickel treated (10 mg/kg b. wt.)
Number of dams	15.00	15.00
Corpora lutea	12.00±1.64	13.53±1.18
Implantation	12.40±0.82	10.93±1.16*
Pre implantation loss (%)	3.33±0.82	19.20±1.16*
Live foetuses	11.83±1.63	9.13±1.16*
No. of resorptions	0.57±0.05	1.80±0.77*
Post implantation loss (%)	4.59±1.63	16.46±1.16*
Foetal weight (g)	0.889±0.16	0.417±0.13*
Foetal crown-rump length (mm)	3.60±0.16	3.41±0.31

* $p < 0.05$ considered to be statistically significant.
Pre implantation loss (%) = (Corpora lutea-implantation/Corpora lutea)×100.
Post implantation loss (%) = (implantation – Live foetuses/ implantation)×100.

surface area of seminal vesicles following exposure to nickel is reduced. Such an effect could adversely effect the secretion from the seminal vesicle which are essential for maintenance of the sperm. Similar type of morphological effect have been observed in seminal vesicle following exposure to the cadmium (Nordberg 1975).

Abnormal sperm morphology has been associated anecdotally with spontaneous abortion (Oehninger *et al.* 1989). The associated abnormalities of sperm flagellar activity may be responsible for fertilization dysfunction (Morales *et al.* 1988) or nickel treatment reduces the fertilizing capacity of spermatozoa (Jacquet 1982). Male mice exposed to nickel sulphate were able to imprignate the unexposed femal and the rate of pregnancy was observed to be comparatively in lower number as compared to unexposed male mice (Deknudt & Leonard 1982). The decrease in fertility was induced in 10 mg/kg group where the increase in abnormal sperm and decrease in epididymal sperm were noticed. This is well supported by the observations of Mestrich (1989) who reported that infertility occurs when the sperm count falls significantly below normal. Thus the observed decrease in male fertility of mice exposed to 10 mg/kg for 35 days may be due to lowered epididymal sperm count. Female mated with controls males had higher implantation. The foetal loss may occur both before and after implantation. The pre implantation loss induced by nickel treatment of males is due to a toxic effect on spermatids and spermatogonia (Jacquet 1982), while post implantation loss may be due to genetic factors (Mathur *et al.* 1978). The increase in pre and post implantation losses, resorbed foetus, decreased foetal weight in the mice mated with the nickel sulphate exposed males may be due to developmental toxicity of this metal.

Acknowledgements

The authors are grateful to the Director, Industrial Toxicology Research Centre, Lucknow and Head, Department of Zoology, Lucknow University, Lucknow for their keen interest in the present studies. One of the authors (RP) is thankful to University Grant Commision, New Delhi for Providing financial assistance as Junior Research Fellow. Mr S.Q. Mehndi and Mrs Poonam Saxena are acknowledged for providing support in analysis of metal on AAS. Thanks are also due to Mr Pramod Kumar Srivastava for technical assistance. The ITRC publication number is 2032.

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