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DNA Fragmentation in Spermatozoa due to Cadmium and Lead Intoxication among Some Egyptian Population

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Abstract The present study aimed to throw the light on cadmium and lead concentrations in seminal plasma among some Egyptian men with infertility history that are likely to be representative of those toxins among the Egyptian general population and to predict the most affected semen parameters by the above metals. In addition, the current study aims to explore the possible correlation of spermatozoa DNA damage with the cadmium and lead metals concentration. Ninety semen samples obtained from patients attending the andrology out-patient's clinic, Mansoura University Hospital. Studied groups include group of idiopathic infertile men (n=46) and group of fertile healthy men (n=44) who matched according to age and residence. Semen analysis results revealed highly statistical significant differences on comparing infertile to fertile groups. There was highly statistical significant increase in cadmium and lead seminal plasma levels on comparing infertile to fertile groups. DNA laddering test revealed 82.6% DNA damage in infertile group comparing to 11.4% in fertile group. The results revealed a positive correlation between both metals level and spermatozoa DNA damage percentage in infertile group. Linear regression analysis revealed that exposure to cadmium affected sperm motility then sperm function while, exposure to lead affected sperm motility then morphology. In conclusion, chronic low-level exposure to environmental toxicants such as cadmium and lead impair male reproductive ability.

Keywords Male Infertility, Cadmium, Lead, Semen Analysis, DNA Laddering

Introduction

Environmental contamination by heavy metals has drawn more concerns because of their potential health hazard impacts on human and animals, especially the reproductive functions. Population is exposed to metals at low concentrations either through supplementation or through intake of contaminated food and water or contact with contaminated soil, dust, or air (CDC, 2005).

Studies of various contaminated exposed wildlife populations suggest that multiple mechanisms contribute to changes in gonadal development, maturation of sperm cells, fertilization, and pregnancy (Guillette and Moore, 2006).

Several metals mainly lead and cadmium are considered reproductive toxicants and human populations could be exposed to heavy metals at trace concentrations (Mendiola et al., 2011)

Cadmium (Cd), a toxic heavy metal element, is widely distributed in the environment and has an exceptionally long biological half-life about 20–40 years resulting in accumulation in the body throughout whole life (WHO, 2000). Exposure to cadmium may account for declining of male fertility as it can induce germ cell apoptosis and decrease daily sperm production (Akinloye et al., 2006; Monsefi et al., 2010). Also, Cd has been linked to poor human semen quality and DNA damage (Telisman et al., 2000; Xu et al., 2003).

Although lead poisoning cases are rare nowadays, chronic lead exposure remains a major public health problem worldwide. Lead (Pb) may adversely affect sperm shape, motility, and DNA integrity (Eibensteiner et al., 2005; Hernandez-Ochoa et al., 2005; Telisman et al., 2007).

However, human data on non-occupational exposure to these metals has been limited or inconsistent across studies. The present study aimed to throw the light on cadmium and lead concentrations in seminal plasma among some Egyptian men with infertility history that are likely to be representative of those toxins among the Egyptian general population and to predict the most affected semen parameters by

the above metals. In addition, the current study aims to explore the possible correlation of spermatozoa DNA damage with the cadmium and lead metals concentration.

Material and Methods

Subjects

The present study was conducted on 90 semen samples obtained from patients attending the andrology out-patient's clinic, Mansoura University Hospital. Studied groups include group of idiopathic infertile men (n=46) with age ranging from 25-40 years and group of fertile healthy men (n=44) who matched according to age and residence. Informed written signed consent was obtained from all participants.

The exclusion criteria

The study excluded infertile men with diabetes, thyroid, or adrenal disorders, genetic disorders related to fertility, those with varicoceles, testicular cancer, bilateral orchiectomy, those taking hormone therapies, cigarette smokers, and those working in metals, fumes, or dust factories.

Methods

1-Semen analysis

In all groups of subjects, fresh semen specimens were collected by masturbation. Subjects collected semen after 2 to 3 days of abstinence from ejaculation. All semen was collected into metal ion–free sterile containers. Semen analysis performed according to World Health Organization (WHO) criteria (WHO, 2010). All semen specimens were allowed to liquefy before analysis. The semen parameters investigated in this study were sperm concentration (million sperm per milliliter), sperm motility, and sperm morphology.

The criteria for normozoospermia were ≥20×106/ml concentration with grade A motility in 25% or grade A+B motility in 50% of spermatozoa and normal morphology in at least 30% of the spermatozoa (WHO, 2010).

2- Sperm Function Testing: Assay of acrosin activity (by gelatin-covered microslides and gelatinolysis)

Gelatin-covered slides were prepared by spreading 20 µl of 5 % gelatin (Merck, Darmstadt, Germany) in distilled water on the slides. The slides were then air-dried, stored at 4 °C overnight and fixed and washed in phosphate-buffered saline (PBS) (Henkel et al., 1995). Semen samples of 20 µl were diluted 1:10 in PBS containing 15.7 mmol/L a-D-glucose. Semen samples were smeared on prepared slides and incubated in a moist chamber at 37 °C for 2 h. The halo (Figure 2A) diameter around any 10 spermatozoa shown to be representative of sperm present in the ejaculate was measured in phase contrast with an eyepiece micrometer. The halo formation rate was calculated per slide as the percentage of spermatozoa showing a halo. One hundred spermatozoa were evaluated. An acrosin activity index was calculated by multiplying the halo diameter by the halo formation rate (Zalata et al., 2004).

3- Spermatozoa DNA Fragmentation Analysis (Daniel et al., 1999):

Spermatozoa were collected after centrifugation and DNA fragmentation was assessed by Enhanced Apoptotic DNA Ladder Detection kit (BioVision Research Products 980 Linda Ista Avenue, Mountain View, CA 94043 USA). DNA ladder was visualized by illumination of short UV wavelength (254 nm) and photographed with camera equipped with 520 nm filter (Figure 1).

4- Determination of Cadmium and lead Levels in Seminal Plasma (Martin et al., 1994):

Analysis was made by using a known weight (0.5) of the fresh samples and wet digestion was conducted using a microwave oven (Milestone mps 1200 mega). The conditions for wet ashing were as follows: the sample was mixed with 6 ml of concentrated nitric acid (65%v/v) and heated with microwave generated from the oven at moderate full power for 15 min. Total content micronutrients were determined in the digested solution using Inductively Coupled Plasma (6000) emission spectrometry (ICP spectrometer; iCAP 6000 series; Thermo Scientific) (Martin et al., 1994).

Statistical analysis

All data were subjected to descriptive and discriminate analyses using the SPSS package (version 16; SPSS Inc., Chicago, IL).

Statistical significant of the difference between parametric data was evaluated with Student's-t-test and computing the correlation coefficient of Pearson. Difference between non-parametric data was evaluated with chi-square and computing the correlation coefficient of spearman. Linear regression analysis used to predict the most affected parameters.

Results

The mean age of the infertile group (n=46) was 35.34±4.45 while the mean age of the fertile group (n=44) was 33.26±6.34. Most of the infertile group cases were working as employee (43.48%), farmers (21.74%), then carpenters and drivers (10.87% for each). As regards the type of infertility, the majority was suffering from primary infertility (69.57%) and the duration of infertility was: 5 (19.56%), 7 (21.74%) and 10 (58.69%) years as shown in Table 1.

Semen analysis results revealed highly statistical significant differences as regards semen volume, sperm function, concentration (mill/ml), motility, and normal morphology (%) on comparing infertile to fertile groups (Table 2). Figure (2A) demonstrated acrosin activity and halo formation around the head in fertile group while, figure (2B) showed poor acrosin activity and absence of halo around the head in infertile group.

There was highly statistical significant increase in cadmium and lead seminal plasma levels in comparison between groups as the levels were 1.63±0.69 and 5.04±2.65 µg/dl for cadmium and lead respectively in fertile group while, 3.41±0.94 and 18.71±6.48 µg/dl for cadmium and lead respectively in infertile group (Table 3).

Spermatozoa DNA fragmentation test revealed 11.4% DNA damage in fertile group comparing to 82.6% in infertile group and chi-square test showed statistical significant difference between both groups as shown in Table 4. Figure 1 demonstrated DNA laddering in infertile group (lane 2) and normal DNA in fertile group (lane 3).

There was significant negative correlation between cadmium seminal plasma levels and sperm concentration while, there was significant negative correlation between lead seminal plasma levels and sperm motility. In addition, there was significant positive correlation between both metals level and spermatozoa DNA damage percentage in infertile group (Table 5 & 6).

Linear regression analysis to predict the common semen parameters that affected by cadmium and lead levels revealed that exposure to cadmium affected sperm motility then sperm function while, exposure to lead affected sperm motility then morphology (Table 7 & 8).

Table (1): Distribution of occupation, type, and duration of infertility in infertile group.

	Infertile group (n=46)	
	Number	**Percentage**
Occupation		
Carpenters	5	10.87
Employee	20	43.48
Farmers	10	21.74
Engineer	1	2.17
Drivers	5	10.87
Medical representative	1	2.17
Others	4	8.70
Type of infertility		
Primary	32	69.57
Secondary	14	30.43
Duration of infertility		
5 years	9	19.56
7 years	10	21.74
10 years	27	58.69

Table (2): Student "t" test statistical analysis of semen analysis parameters in the studied groups.

Semen analysis		Fertile group (n=44)			Infertile group (n=46)			t	*p*
		Mean	**SD**	**Median**	**Mean**	**SD**	**Median**		
Semen volume (ml)		4.25	0.57	4.00	2.84	0.29	2.80	14.71	<0.001*
Sperm function	Acrosin index	13.2	2.3	12.9	5.2	2.1	5.6	10.58	<0.001*
	Halo	18.5	2.6	18.7	12.2	1.8	12.1	13.42	<0.001*
	Halo %	71.5	8.1	71.0	42.3	16.6	47.0	10.58	<0.001*
Concentration (mill/ml)		56.1	19.6	56.3	14.6	8.7	12.9	13.04	<0.001*
Motility	Grade A	33.8	5.3	35.0	13.2	7.4	13.5	15.15	<0.001*
	Grade B	14.5	3.2	15.0	9.8	4.9	10.0	5.41	<0.001*
	Grade A+ B	48.3	6.4	49.0	23.0	8.0	23.0	16.45	<0.001*
	Linear index	84.5	5.9	85.5	62.3	12.6	65.0	10.65	<0.001*
	Linear velocity	36.8	10.4	34.9	14.9	7.9	12.8	11.25	<0.001*
	Velocity	43.5	11.7	43.8	22.9	9.5	22.1	9.21	<0.001*
Morphology (normal)		43.3	9.3	43.0	18.2	10.8	20.0	11.81	<0.001*

**Significant if P < 0.05. If p is significant, it means there is a difference with statistical significance between both groups.*

SD: standard deviation.

Table (3): Student "t" test statistical analysis of seminal cadmium and lead levels in the studied groups.

Groups	Cadmium (µg/dl)	Lead (µg/dl)
Fertile group (n= 44)		
Mean	1.63	5.04
SD	0.69	2.65
Median	1.66	4.60
Min.	0.49	0.87
Max.	2.74	9.63
Infertile group (n= 46)		
Mean	3.41	18.71
SD	0.94	6.48
Median	3.25	18.06
Min.	1.80	6.73
Max.	4.89	29.68
"t" test	10.24	18.47
***P* value**	<0.001*	<0.001*

**Significant if P < 0.05. If p is significant, it means there is a difference with statistical significance between both groups.*
SD: standard deviation.
Min.: minimum value.
Max.: maximum value.
n.: number of cases.

Table (4): Chi-square statistical analysis of positive DNA damage in studied groups.

	DNA damage	Chi-square	*p*
Fertile group (n=44)			
Negative DNA damage	n=39 (88.6%)		
Positive DNA damage	n=5 (11.4%)	39.30	<0.001*
Infertile group (n=46)			
Negative DNA damage	n=8 (17.4%)		
Positive DNA damage	n=38 (82.6%)		

**Significant if P < 0.05.*
If p is significant, it means there is a difference with statistical significance between both groups.
n.: number of cases.

Table (5): Pearson's correlation between seminal levels of cadmium and lead and semen parameters in infertile group.

Variables	Sperm Function			Sperm Concen-tration (mill/ml)	Sperm Motility						Sperm Morph-ology
	Acrosin index	Halo	Halo %		Grade A	Grade B	Grade A_B	Linear index	Linear velocity	Velocity	
Cadmium											
Pearson correlation	-.170	-.139	-.134	-.364	-.070	-.166	-.164	-.014	.022	.068	-.035
P value	.258	.357	.374	.016*	.644	.271	.276	.928	.883	.655	.816
n	46	46	46	43	46	46	46	46	46	46	46
Lead											
Pearson correlation	.030	.013	.022	-.160	.021	.043	.045	.107	-.361	-.384	.025
P value	.842	.929	.885	.306	.890	.776	.765	.481	.014*	.008*	.868
n	46	46	46	43	46	46	46	46	46	46	46

**Correlation is significant if P < 0.05.*
If p is significant, it means there is a correlation with statistical significance between both variables.
n: number of patients

Table (6): Spearman's correlation between seminal levels of cadmium and lead and spermatozoa DNA damage in infertile group.

Spearman's Correlation	Spermatozoa DNA damage
Cadmium seminal levels	
r	.482
P value	(<0.001*)
Lead seminal levels	
r	.521
P value	(<0.001*)

**Correlation is significant if P < 0.05.*
If p is significant, it means there is a correlation with statistical significance between both variables.
r=Correlation coefficient

Table (7): Linear regression coefficient for seminal cadmium levels and semen parameters in infertile group.

Semen parameters		B	SEE	*p*
Sperm function	Acrosin index	.354	.195	<.044*
	Halo	-.248	.126	<.043*
	Halo %	-.057	.026	<.034*
Concentration (mill/ml)		.004	.006	.480
Motility	Grade A	-.037	.013	<.007*
	Grade B	-.030	.025	.233
	Linear index	.012	.016	.457
	Linear velocity	-.037	.060	.543
	Velocity	.016	.048	.742
Morphology (normal)		-.019	.011	.086
Constant		8.633	2.152	
Dependent variable: cadmium level				

** Significant if P < 0.05*
B: regression coefficient.
SEE: standard error of estimates.
P Significant: it means the variable has a strong risk effect on the semen parameters.

Table (8): Linear regression coefficient for seminal lead levels and semen parameters in infertile group.

Semen parameters		B	SEE	*p*
Sperm function	Acrosin index	.557	1.163	.633
	Halo	-.763	.748	.311
	Halo %	-.134	.156	.394
Concentration (mill/ml)		-.059	.034	.085
Motility	Grade A	-.232	.079	<0.005*
	Grade B	-.019	.146	.895
	Linear index	-.001	.096	.989
	Linear velocity	-.270	.356	.451
	Velocity	.269	.289	.355
Morphology (normal)		-.120	.065	<0.049*
Constant		38.863	12.811	
Dependent variable: lead level				

** Significant if P < 0.05*
B: regression coefficient.
SEE: standard error of estimates.
P Significant: it means the variable has a strong risk effect on the semen parameters.

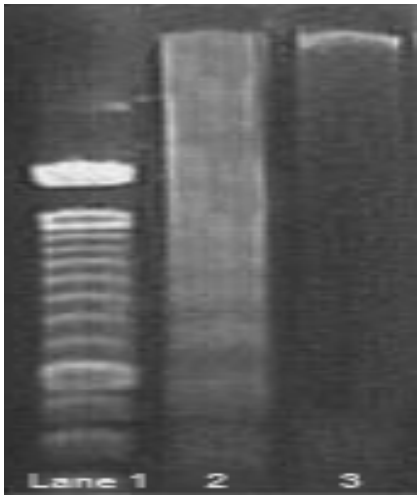
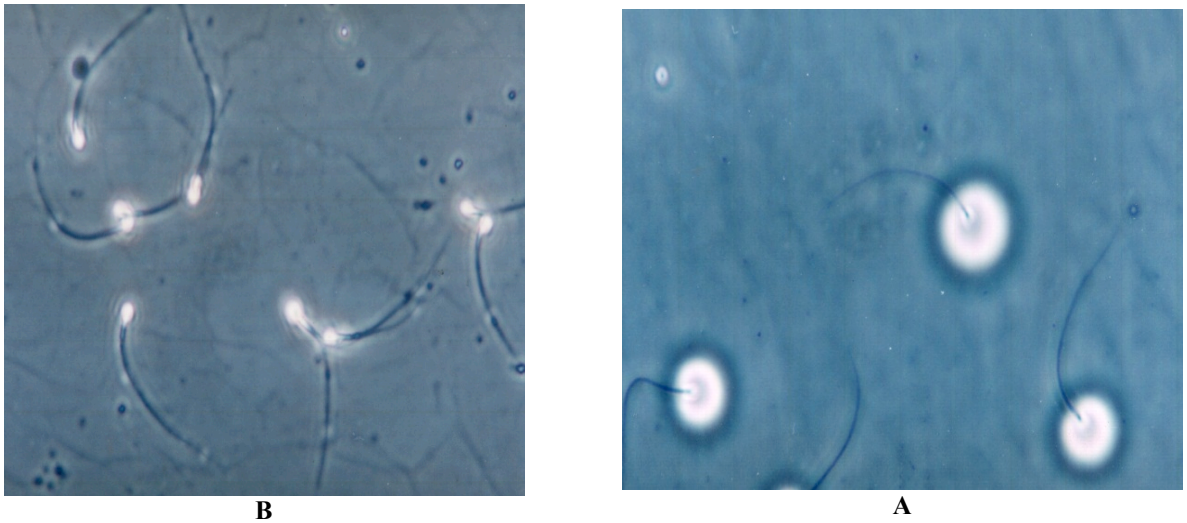


Figure (1): Analysis of genomic DNA fragmentation in human spermatozoa (sperm pellet 10 x 106 cells). Lane 1: 100-bp ladder (GIBCO-BRL). Lane 2 (infertile group) showed DNA fragmentation (DNA ladder) and lane 3 (fertile group) showed no DNA fragments (no DNA ladder).



B **A**

Figure (2): Photomicrograph of human spermatozoa after 2 hours incubation on gelatin slides showing good acrosin activity and halo in fertile group (A) and poor acrosin activity and no halo in infertile group (B).
Phase contrast microscope (X500)

Discussion

The present study aimed to throw the light on cadmium and lead concentrations in seminal plasma among some Egyptian men with infertility history that are likely to be representative of those toxins among the Egyptian general population and to predict the most affected semen parameters by the above metals. In addition, the current study aims to explore the possible correlation of spermatozoa DNA damage with the cadmium and lead metals concentration.

In the current study, most of the infertile group cases were working as employee, farmers, then carpenters and drivers. As regards the type of infertility, the majority was suffering from primary infertility and the duration of infertility commonly was 10 years (Table 1).

Semen analysis results in the present work revealed highly statistical significant differences as regards semen volume, sperm function, concentration (mill/ml), motility and normal morphology (%), comparing infertile to fertile groups (Table 2).

Besides, the present study showed highly statistical significant increase in the level of cadmium and lead in seminal plasma, comparing infertile to fertile groups (Table 3).

Some human studies have reported declines in semen quality associated with elevated cadmium level in blood (Telisman et al., 2000; Akinloye et al., 2006) and its elevated level in semen (Pant et al., 2003; Mendiola et al., 2011).

On the other hands, Kasperczyk et al. (2002) stated that seminal plasma cadmium levels have been

reported to be unrelated to semen parameters and fertility status.

While, Xu et al. (2003) and Wu et al. (2008) observed an inverse correlation between cadmium level and sperm concentration.

In accordance, Benoff et al. (2009) showed an inverse correlation of seminal plasma cadmium levels with semen parameters in infertile men from Rochester, New York, USA and observed that seminal cadmium levels elevated specifically in infertile patients and its levels associated with decreased semen quality.

Lead is widely used in acid battery, plant refinery, smelter, fuel combustion industry, printing press and automobile exhaust where tetraethyl lead acts as anti-knocking agent. Toxicity is manifested in male reproductive system by deposition of lead in testes, epididymis, vas deferens, seminal vesicle and seminal ejaculate (Roy-Chowdhury, 2009).

In the current study, linear regression analysis to predict the common semen parameters affected by the cadmium and lead levels revealed that exposure to cadmium affected sperm motility then sperm function while, exposure to lead affected sperm motility then morphology (Tables 7 and 8)

In agreement with the present study, Telisman et al. (2007) reported non-occupational lead exposure, measured as blood lead levels, to be associated with increased immature sperm and percentage of pathologic, wide, and round sperm in a study of Croatian men.

Another study in Mexico found that lead measured in spermatozoa or seminal fluid, was associated with decreased semen quality (Hernandez-Ochoa et al. 2005).

However, for metals that are stored in the body, such as lead, a single measure is likely a reliable marker of exposure over months or years (Egeghy et al., 2005). The reliability of a single semen sample to represent a man's semen quality over a longer period of time continues to be debated, although two recent reports have provided limited evidence that one sample may be adequately representative of semen quality over several weeks in large epidemiologic studies done in Andrology Unit, Department of Internal Medicine at the University of L'Aquila, Italy (Francavilla et al., 2007; Stokes-Riner et al., 2007).

In the present study, DNA fragmentation laddering test which is a method to distinguish toxic cell death revealed more DNA damage in infertile group comparing to fertile group (Table 4).

Currently, limited information is available about the genetic contribution to sensitivity or resistance to cadmium. Although dietary intake of cadmium is higher in men than women (Watanabe et al., 2004), cadmium retention is higher in women than in men; for example, blood, urine, and kidney cadmium are elevated in women compared with men (Nishijo et al., 2004; Vahter et al., 2007).

In accordance, Bu et al. (2011) observed that, oral administration of cadmium (4 mg/kg for 2 weeks) in male mice significantly induced seminiferous epithelium damage in the early stage of spermatogenesis. Increased apoptotic germ cells were found in seminiferous tubules, mainly consisting of round spermatid and elongate spermatid. Moreover, higher cadmium treatment resulted in severe necrosis of the seminiferous epithelium. This result indicated that the degree of germ cell damage was related with the dose of cadmium exposure.

Kasuba et al. (2004) suggested that low doses of lead acetate cause detectable genome damage in experimental study.

Conclusion

Since the infertile group in the current study had no histories of occupational exposure to heavy metals or metal poisoning, the study results concluded that chronic low-level exposure to environmental toxicants such as cadmium and lead could impair male reproductive ability.

Recommendation

The governments should do efforts to educate the public to hazards of environmental cadmium and lead exposure as they may affect fertility. Cadmium is a component of tobacco smoke (passive or active) so avoid smoking in enclosed spaces like inside the home or car in order to limit exposure. Use all safety precautions to avoid carrying cadmium-containing dust home from work on clothing, skin, hair, or tools. A balanced diet (healthy diet, including lots of protein, iron and vitamin C) can reduce the amount of cadmium and lead taken into the body from food and drink.

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الملخص العربي

تكسير الحمض النووي في الحيوانات المنوية بسبب التسمم بالكادميوم و الرصاص لدى بعض السكان المصريين

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استهدفت هذه الدراسة إلقاء الضوء علي تركيزات الكادميوم والرصاص في البلازما المنوية لدى بعض الرجال الذي لديهم تاريخ مرضي للعقم والتي من المحتمل أن تكون هذه التركيزات ممثلا لتلك السموم بين المصريين وكذلك التنبؤ بأكثر عناصر السائل المنوية المتضررة من التعرض للكادميوم والرصاص. وبالإضافة إلى ذلك، فإن الدراسة الحالية تهدف إلى استكشاف ارتباط محتمل بين تكسير الحمض النووي و تركيز المعادن المذكورة. وقد أقيمت الدراسة علي تسعين عينة من السائل المنوي الذي تم الحصول عليه من بعض المرضي المترددين علي عيادة أمراض الذكورة بمستشفي جامعة المنصورة وتناولت الدراسة مجموعتان: مجموعة العقم وتشمل46 مريضاً من المرضي المترددين علي العيادة الخارجية لمرضي الذكورة والعقم، بمستشفي المنصورة الجامعي، ومجموعة من الرجال الخصبة وتشمل 44 شخص ممن يتناسبوا مع مجموعة العقم وفقا للسن ومكان المعيشة.

وكشفت نتائج تحليل السائل المنوي فروق ذات دلالة إحصائية عالية بمقارنة مجموعة العقم بمجموعة الرجال الخصبة. وكانت هناك زيادة ذات دلالة إحصائية عالية في مستويات الكادميوم والرصاص في البلازما المنوية وذلك بمقارنة مجموعة العقم بالمجموعة الرجال الخصبة. وكشف اختبار الحمض النووي السلمي أن هناك نسبة مئوية 82.6 تكسير بالحمض النووي في مجموعة العقم مقارنة بنسبة مئوية 11.4 في مجموعة الرجال الخصبة. وقد أظهرت النتائج علاقة إيجابية ذات دلالة إحصائية بين مستويات المعادن على حد سواء ونسبة التكسر بالحمض النووي في مجموعة العقم. وكشف تحليل الانحدار الخطي أن التعرض للكادميوم يؤثر بدرجة عالية علي حركة الحيوانات المنوية ثم علي وظيفة الحيوانات المنوية، في حين أثبتت الدراسة أن التعرض للرصاص يؤثر علي حركة الحيوانات المنوية ثم علي نسبة الأشكال الطبيعية للحيوانات المنوية. ونستخلص من الدراسة أن التعرض المزمن لمستويات منخفضة من المواد السامة البيئية مثل الكادميوم والرصاص يضعف القدرة التناسلية للذكور.

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