Zagazig J. Pharm. Sci. December 1996
Vol. 5, No. 2, pp.13-20

ISSN 1110-5089

EFFECT OF NICKEL ADMINISTRATION ON SOME BIOCHEMICAL PARAMETERS AND OVARIAN HORMONES IN FEMALE RABBITS

Abdel-Mohsen M. Hamam, Magda M. El-Tohamy and Gamal A. Ali

Department of Animal Reproduction and Artificial Insemination,
National Research Center, Dokki, Cairo, Egypt.

ABTRACT

This study was designed to clarify the toxic effect of low and high doses of nickel on reproductive functions of immature rabbits treated with PMSG ,with trials to restrict the harmful effects of nickel administration .Thirty seven immature female New Zealand rabbits were used for this purpose , divided into five groups Group I , kept as control ; group II, administered orally low doses of nickel (200 ug/animal) , group III , were given high doses of nickel(1000 ug/animal) ; group IV , were given low doses of nickel with 50 ug manganese sulphate .The treatment with nickel and/or manganese were continued for 8 weeks , five times per week .Group V, not treated with PMSG and kept as control .All groups(except group V)were treated with 200 I.U. pregnant mare serum gonadotrophin (PMSG) before decapitation of animals. The results showed that both nickel doses induced inhibitory effect on the ovarian activity either in the ovarian weight or in the number of growing follicles and the total ovarian response . Estradiol-17β was significantly increased in both the plasma and ovarian tissues after treatment with low doses of nickel and decreased significantly in the ovarian tissue of high dose nickel group . Progesterone concentration was significantly decreased in the plasma of all treated groups and in the ovarian tissues of groups III and IV only, compared with control group . Thyroxine (T4) recorded a significant decrease in group III only .Fractionation of protein showed only elevation in gamma globulins with low dose levels of nickel , while other types of globulins were not affected . Regarding the blood picture , WBCs were affected significantly in all experimental groups , while RBCs decreased after low nickel dose treatment only .A highly significant increase of nickel in the plasma and ovarian tissues in group II & III ,was recorded and manganese increased in groups III & IV .Copper level was significantly decreased in both plasma and ovarian tissues in groups II and III . It is concluded that, nickel treatment inhibited the ovarian activity and disturbed the ovarian steroid hormones release and/or biosynthesis , and may have a stimulatory effect on the immune system in low doses .

INTRODUCTION

Nickel enters ground water and surface water from dissolution of rocks, soils ,metal plating plants , from biological cycles; from industrial processes and water disposable. Electroplating waste water contains high levels of chromium, copper ,nickel and zinc [1]. The toxicity of nickel compounds to aquatic organisms is markedly influenced by the physico- chemical properties of water . In soft waters with low calcium concentrations, the lethal concentrations of nickel compounds were decreased .

Studies of acute poisoning in workers and experimental animals show that inhaled Ni $(Co)_4$ vapor is rapidly absorbed via the lung and enters erythrocytes, where the compound undergoes conversion to Ni^{2+} and carbon monooxide[2]. The alimentary absorption of Ni averaged 27% of the dose ingested in water on overnight fast [3] , traces of Ni^{2+} in hemodialysis fluid are absorbed into the plasma compartment, owing to the chelating action of plasma albumin .

The mechanism of the toxic action of metals on fish is variable . Most of the metals have great affinity for amino acids and the SH groups of proteins , they act as enzyme poisons . The toxicity of metals to fish is significantly affected by the form in which they occur in water . The ionic forms of metals or simple inorganic compounds are more toxic than complex inorganic or organic compounds .Various workers have attempted to describe different mechanisms of action of heavy metals on gonads . Kumar and Pant[4] have attributed direct effect of heavy metals on fish gonads , besides their possible action through pituitary gland ; Sangalang & O'Halloran [5] have opined alterations in steroid synthesis . Other investigators [6] suggested inhibition of the action of pituitary gonadotrophs and somatotrophs .An over abundance of one trace elemenet can interfere with the metabolic utilization of another element present in normal or marginal concentrations [7] .

Large variety of data regarding toxic effects of nickel on mammals have been reported . Nickel refinery workers had increased mortality from lung or sinonasal cancers . In human populations exposed to Ni and chromium compounds , chromosomal abberations have been reported. Biochemical as well as histopathological alterations arising from Ni exposure in mammals have also been documented [8] . The chronic effects of exposures to Ni compounds primarily affect the immune system and the respiratory tract [9]. Hyperglycaemia and glucosurea develop following exposure to $Ni(Co)_4$.

The effect of nickel on reproduction system was studied by many investigators. Significant histopathological alterations were observed in the ovaries of fish exposed to sublethal concentration of nickel. The prominent changes were-occurrence of atretic oocytes and increase in interfollicular spaces [10].

However, reduced reproduction has been found to be a more sensitive measure of heavy metal toxicity than survival [1,11]. A significant increase in the frequency of sperm micronucleus abnormalities after exposure to 6 days or 5 weeks treatment of nickel compounds [12].

Some authers[13] suggested that the low developmental ability of pronuclear stage of rat embryos may be mainly due to deleterious effect of heavy metal ions and hypoxanthine. Scanty data are available on the effect of nickel exposure on reproductive potentials of female rabbits, therfore the aim of the present work was to investigate the effect of oral administration of different doses of nickel chloride on ovarian activity, ovarian steroid hormone patterns and some biochemical and haematological changes, with trials to study the effect of treatment with manganese sulphate to elevate or not the toxic effect of nickel on reprodutive function.

MATERIAL AND METHODS

Animals :

Thirty seven virgin female white New Zealand rabbits were used. Maintained in individual wire cages, given limited food and water, reared in The Animal House, National Research Centre, Cairo.

Experimental design :

The virgin does (immature female rabbits)were randomly divided into 5 groups, each of 8 animals for groups I,II, III and IV and 5 animals for group V. The first group was left as control, the second group drenched low doses of nickel chloride (200 µg/ animal), the third group treated with high doses of nickel chloride (1000 µg/ animal). While the 4th group drenched low doses of nickel chloride (200 µg) and manganese sulphate (50 µg/animal). The animals were treated 5 times per week for 8 weeks. The fifth group, not treated with PMSG or HCG and kept as control.

All groups (except group V)were treated with 200 i.u. pregnant mare serum gonadotrophin (PMSG, Folligon®, Intervet, holland), in followed by injection of 200 i.u. Human chorionic gonadotrophin (HCG, Pregnyl®, Organon, Holland), 48 hrs after initiation of PMSG treatment or 2hrs after mating (female accepted male). then, 24-48 hrs later the animals were decapitated, where the ovaries were collected, kept in saline (0.9%Nacl) weighted and examined for determination of the total ovarian response (follicles, corpora haemorrhagica and corpora lutea). Some ovaries were immediatly excised, trimed from connective tissues, weighed, minced and homogenized in polytran homogenizer, diluted with distilled water. The ovarian homogenates were centrifuged and the supernatant was analyzed for steroid hormones assay and minerals.

Blood samples :

Blood samples were collected during decapitation of rabbits in 3 tubes containing either EDTA (for haematological examination), heparin (for separation of plasma) or whole blood (to separate blood serum for electrophoresis). Aliquots of blood were collected freshly on EDTA for haematological pictures (RBCs, WBCs, Hb, HCT and MCV) using Cell Counter Serono Diagnostic 190 T.

Blood plasma was collected in heparinized tubes and kept at -20°C, until analysed for hormonal assay and trace & heavy metals.

Progesterone and estradiol-17β were determined using radioimmunoassay according to the methods of Abraham[14] and Allen & Redshaw [15]. Triiodothyronine (T3) was determined according to the method of Eastman et al.[16] and Thyroxine (T4) was measured in the plasma using RIA technique according to the method of Chopra et al[17].

Electrophoretic patterns were done using polyacrylamide gel electrophoresis [18, 19].

Nickel, manganese, zinc and copper were determined in the plasma and ovarian tissues by using atomic absorption spectrophotometry [20].

Statistical analysis :

The data were analysed statistically according to Tallarida and Murray [21].

RESULTS AND DISCUSSION

Effect of low and high doses of nickel on ovarian activity :

In the present study, all virgin does (except group V)were treated with 200 i.u. pregnant mare serum gonadotrophin(PMSG) to augment the follicular growth, followed by injection of Human chorionic gonadotrophin (HCG) 2 hrs after mating to induce ovulation in virgin does.

As shown in table (1) injection of PMSG, 4-5 days before decapitation of rabbits stimulated the ovarian growth and increase the ovarian weights (0.85± 0.08 gm/ ovary, $p<0.01$) compared with rabbits in group V,which is not treated with PMSG & HCG (0.28 ± 0.02 gm / ovary wet weight) where the ovaries of this control group did not show prominant follicular growth. The treatment resulted also in an increase in the number of growing follicles (38.9± 2.28 follicle/ ovary), number of ovulated follicles (17.6±2.37 C.H + C.L/ ovary) and in the total ovarian response (56.5 ± 4.1).

Administration of low doses of nickel chloride (group II) over 8 weeks to female rabbits decreased significantly the ovarian weights (0.448 ±0.03 g vs. 0.85 ± 0.08 gm/ ovary), number of growing follicles (18.08 ± 1.77 follicle/ ovary, $P < 0.01$, number of C.L. (0.8 ± 0.39 C.L./ovary, $p < 0.01$) and the total ovarian response (31.5 ± 0.75 F + CH + CL/ ovary). This effect

ISSN 1110-0389

of nickel on the ovarian activity may be referred to changes in the hormonal environment or steroid balance in the follicular fluid or tissues [14]; or may be due to direct effect of heavy metals on ovaries, besides their possible action through pituitary gland [15] Which may lead to alterations in steroid synthesis or through inhibition of the action of pituitary gonadotrophins[16]. Some authors reported a histopathological alterations in the ovaries exposed to sublethal concentration of nickel. These changes are mainly occurrence of atretic oocytes and increase in inter follicular spaces [16].

Table(1) Effect of administration of low and high doses of nickel on ovarian weight , total ovarian activity of virgin does treated with PMSG

Parameters	Groups of female rabbits			
	Control (Group I)	Low dose nickel (Group II)	High dose nickel (GroupIII)	Lowdose+Mn (Group IV)
Ovarian weight (gm)	0.851±0.078	0.448±0.027***	0.653±0.046*	0.462±0.032***
Follicles	38.9±2.28	18.08±1.77***	20.28±1.6**	15.7±2.02***
C. haemorrhagica	12.1±2.5	13.91±1.74	12.5±2.13	5.84±0.86*
Corpora lutea	5.22±1.01	0.84±0.49***	6.04±1.19	1.8±1.2*
Ovulation(%)	17.6±2.37 (31.1%)	13.4±1.72 (42.6%)	18.5±1.8 (69.9%)	7.1±1.2*** (17.37%)
T. ovarian response	56.5±4.1	31.5±0.75***	38.85±2.98*	22.8±1.89***

* P< 0.05 ** P<0.01 *** P<0.001

Table(2): Hormonal levels in plasma and ovarian tissues of female rabbits exposed to low and high levels of nickel .

Parameters	Groups of rabbits/levels of hormones			
	Control (Group I)	Low dose nickel (Group II)	High dose nickel (GroupIII)	Lowdose+Mn (Group IV)
Estradiol-17B	173.11±25.47	280.57±63.69*	155.79±34.51	187.95±30.40
Estradiol(pg/gm)	2270.11±734.94	4692.09±443.3*	93.32±12.85**	1565.71±540.97
Progesterone(ng)	13.53±2.82	5.38±0.99**	4.46±1.4**	3.32±0.53**
Progest.(ng/gm)	3275.98±626.1	3252.01±527.8	1289.52±259.2**	1082.41±262.8**
T3 (ng/ml)	137.93±20.08	131.68±21.65	131.21±20.67	136.82±11.39
T4(ng/ml)	2.25±0.33	1.88±0.36	1.41±0.26*	2.13±0.29

* P< 0.05 ** P<0.01

Table(3): Protein fractionation of serum on polyacrylamide of female rabbits treated with low and high doses of nickel or manganese .

Animals	Fractionation of plasma protein			
	Globulins			Albumin
	Alpha (α)	Beta (β)	Gamma (γ)	
Control(I)	6.25±1.92	13.69±4.49	3.49±1.23	20.53±3.211
Low dose Ni(II)	11.04±2.05	8.42±1.36	10.67±1.94*	16.32±3.66
High dose Ni(III)	9.26±1.27	11.77±1.92	5.01±0.90	24.54±5.68
Low dose Ni+Mn (group IV)	9.432±1.49	6.26±1.33	7.84±1.72	16.08±2.15

* P<.0.05

Table(4) Blood picture of female rabbits after low and high doses of nickel and/or Mn

Animals	Haematological picture of female rabbits				
	WBC's	RBC's	HB	HCT	MCV
Control(I)	8.2910.33	6.34440.29	14.56±0.37	42.78±0.85	67.88±2.77
Low dose Ni(II)	15.38±1.75**	5.16±0.37*	14.0±0.57	43.84±1.97	85.74±3.57**
High dose Ni(III)	15.11±1.71**	5.97±0.28	14.57±0.57	41.67±2.56	69.5±0.29
Low dose Ni+Mn (group IV)	12.05±1.44*	5.67±0.32	13.15±0.88	40.8±2.27	70.5±2.63

Table(5): Concentrations of nickel, manganese, zinc and copper (umol/l) in the plasma and ovarian tissues of female rabbits exposed to nickel treatments

Groups	Levels of minerals(umol/l)							
	Plasma				Ovarian tissues			
	Ni	Mn	Zn	Cu	Ni	Mn	Zn	Cu
Control Gr.I	0.071±0.03	1.32±0.02	27.06±0.18	36.68±0.89	6.39±1.55	7.03±0.86	137.03±21.09	37.51±1.54
Low Ni dose Gr.II	0.13**±0.01	1.64±0.22	17.23**±1.27	15.39**±0.95	17.06**±2.09	13.12**±1.66	94.82**±2.37	16.32**±2.46
High Ni dose Gr.III	0.57**±0.06	4.79**±1.15	22.36±0.89	15.14**±0.89	10.95±1.62	9.49±0.60	107.66±2.87	12.28**±1.50
Low Ni+Mn Gr.IV	0.25±0.03	12.27**±2.45	22.74±0.73	23.37**±1.37	8.32±0.61	19.82**±1.94	79.71**±4.14	29.02**±3.31
Control(2) Gr.V.	0.166±0.05	0.65±0.07	27.06±0.18	37.0±1.29	8.31±0.78	--------	--------	--------

Gr.V : control rabbits not treated with PMSG

*p<0.05 **p<0.01 -------- Not determined

Morcover ,administration of high doses of nickel to rabbits inhibited significantly the follicular development (20.28 ± 1.6 follicle / ovary , p < 0.01) and the total ovarian response (38.85 ± 2.98 /ovary , p < 0.01) with remarkable elevation in the ovulation rate (47.79 %) than control group (31.15 %) .

Treatment with low doses of nickel and manganese (group IV) , did not improve the condition but aggrevate the inhibitory action on the ovarian activity, represented by a decrease in the ovarian weight (0.462 ± 0.03 gm / ovary), number of developed follicles (15.7 ± 2.02 follicle / ovary), ovulation rate (7.1 ±1 1.2 /ovary) and in the total ovarian response (22.8 ± 1.89, p < 0.01) . The addition of manganese sulphate with low doses of nickel did not improve the inhibitory actions of nickel on ovarian activity ,and this may be due synergistic action between nickel and manganese on the ovarian level. Many authors found that an over abundace of one trace element can interfere with the metabolic utilization of another element present in normal or marginal concentrations[7].

Effect of nickel on some hormonal levels and protein fractions :

The results in table (2) showed that the level of plasma estradiol-17β is affected significantly with low dose nickel treatment, where it reached 280.57± 63.69 pg / ml, p < 0.01, compared with control group 173.11 ± 25.47 pg/ml. The concentration of estradiol-17β in

ISSN 1110-5089

ovarian tissue was also increased (4692.09 ± 443.3 pg / ml , $p < 0.05$) .

A highly significant depletion of estradiol-17β in ovrian tissue (93.32 ± 12.85 pg/ ml , $p < 0.01$) was recorded after treatment with high doses of nickel chloride, without significant changes in the plasma .

Increased levels of estradiol-β in plasma and ovarian tissues due to administration of low doses of nickel may be due to changes in the steroid balance in the follicular fluid or tissues [22] or may be due to the effect of heavy metals on metabolic and enzymatic activity [23] .

Plasma progesterone concentration was significantly decreased in animals in groups II (5.38 ± 0.99 ng /ml , $p < 0.01$); III (4.46 ±1.41 ng / ml, $p < 0.01$) and IV (3.19 ± 0.53 ng/ ml, $p < 0.01$) .Whereas, progesterone concentration in the ovarian tissue dcereased significantly in groups 3 and 4 , where it averaged 1289.52 ± 259.25 and 1082.41 ± 262.8 ng / gm of tissues, $p < 0.01$, respectively . But with higher doses of nickel a highly significant decrease in the tissue level of estradiol and progesterone was observed, which can be attributed to the presence of large numbers of atretic follicles [8] or due to alterations in steroid synthesis [5] and can be returned to stressful condition from nickel administration with or/ without manganese which alter the pattern of secretion of some reproductive hormones.

The level of triiodothyronine (T3) was not varied significantly between the treated and control groups as shown in table (2) , whereas ,Thyroxine (T4) decreased significantly in animals received low doses of nickle (1.41 ± 0.26, $p < 0.05$) .This action may be due to the great affinity of heavy metals for amino acids and SH groups of proteins .

At the moment ,no data are available on the relationship between the concentration of nickel in biological media and the risk of reproductive potential and cancer. Biological monitoring of nickel may therefore not be of use as a means of risk estimation but only as an indicator of exposure .
However, concentration of nickel in plasma or serum have been successfully used as an indicator of poisoning. The state of nickel in blood are conflicting, there are three nickel fractions in blood: an amino acid bound fraction which is mostly in the form of nickel-L-histidine complex: nickel bound to albumin and nickel bound to α-macroglobulin, exchange and transfer of nickel between L-histidine and albumin appears to be mediated by a ternary complex in the form of albumin-nickel-L-histidine . This is how nickel appears to be transported in blood in rabbits [24].

Fractionation of serum proteins were done using polyacrylamide gel electrophoresis , the results are shown in table (3) . A non- significant elevations in the level of α-globulin were recorded in treated groups. Whereas, β globulins showed a non-significant decrease in all treated groups compared with control group . A highly significant increase in the level of γ -globulin was recorded in rabbits exposed to low doses of nickel , it averaged 10.67 ± 1.94 , $p < 0.05$). These increases in γ-globulins may be due to the effect on the immune system .

The chronic effects of exposures to nickel compounds primarily affect the immune system and the rate of synthesis and volume of distribution of many plasma proteins [9], altered by steroids , either endogenous or exogenous [25] .

Effect of nickel on haematological picture :

As shown in table (4), the blood picture revealed a highly significant elevation in WBCs counts in all treated groups either received low and high doses of nickel (15.38 ± 1.75 x 10^3 and 15.1 ± 1.71 x 10^3, $p < 0.01$) or low nickel doses with manganese (12.05 ± 1.44 x 10^3 , $p < 0.05$). Sunderman et al. [9] reported that the chronic effects of exposures to nickel compounds primarily affect the immune system.
In addition, a significant drop in the RBCs count was obtained in animals received low doses of Ni (5.16 ± 0.37 x 10^6 , $p < 0.05$) . No significant alterations in the levels of Hb and HCT were recorded in all treated groups , but MCV showed a highly significant increase in rabbits treated with low doses of Ni (85.74 ± 3.97, $p < 0.01$) . The experiments showed that nickel carbonate is rapidly absorbed and enters the RBCs, where the compound converts to Ni^{2+} and $Co^{(2)}$.

Levels of Nickel, manganese ,zinc and copper in the plasma and ovarian tissues :

As shown in table (5) concentrations of nickel in both plasma and ovarian tissues are increased significantly ($p < 0.01$) in low dose level of nickel treated group .Moreover, nickel increased significantly ($p < 0.001$) in plasma only at high dose level

treatment. Regarding manganese in plasma, it increased significantly ($p < 0.01$) from 1.325 ± 0.02 in control to 4.79 ± 1.15 and 12.27 ± 2.45 umol / l in nickel high dose group (group III) and in low dose and manganese treated group (group IV), respectively.

On the otherhand, manganese was found to increase in low dose nickel treated group ($p < 0.05$) and at low dose nickel treated group with manganese ($p < 0.001$). Zinc was found to decrease in plasma in low dose nickel group (17.23 ± 1.27 umol /l, $p < 0.01$) and in ovarian tissue to 79.71 ± 4.14 umol / l , $p < 0.05$) in low dose treated group with manganese .

Copper also decreased significantly in both low and high doses of nickel in plasma and ovarian tissues ($p < 0.001$) and in ovarian tissue only in group IV ($p < 0.05$)

In the groups received low and high nickel doses, there were significant elevations of nickel concentration in the plasma . At the same time significant reduction of zinc and copper concentrations were also observed . It is proposed that much of the known toxicity of nickel may be rationalized by its interference with the normal biochemical and physiological roles of zinc and copper[28].

The biological role of zinc is very extensive and ranges from structural stabilization (e.g. of proteins), to the control , not only of communication between cells but also of the structure and inlegisity of individual cells, to enzyme activation; to the regulation of many fundamental intracellular processes[27, 28].

An important question thus arises whether intracellular and extracellular Ni^{2+} levels reach concentrations high enough to compete with zinc or copper . Similarly , are the intercellular and especially the nuclear levels of nickel attained of sufficient magnitude to make replacement of zinc or copper , a realistic toxicological pathway . These metal ions are especially important in replication, transcription , translation and repair processes[29] .

The replacement of Zn by nickel and Cu appears to be associated with the deactivation of critical enzyme and many important hydrolytic enzymes including the DNA/RNA polymerases[32] . It is probable that the interactions at low or high Ni levels, interface with proteins , enzyme and metal ions.

Metal-metal interactions, especially interactions with essential elements are clearly a key aspect of metabolic carcinogenesis . This includes the inhibition of nickel-induced sarcomas by excessive manganese[30] or magnesium[31]

The results of the present study indicate that the probable derangement in manganese metabolism brought about by Mn supplementation can have a marked enhancing effect on reduction of Zn and Cu levels in both plasma and ovaries. Mn supplementation also decreased levels of Ni in both plasma and ovaries .

In conclusion , as manganese played a role in regaining some of the tested parameters when added with low dose of nickel , this may raise the importance of it as corrective or protective agent in nickel poisoning , and at the same time since it is not of value in some others . This may shed light on the importance of studying the other factors like offering more time to manganese to work either on different enzymes or metabolites involved in the process . On the other hand, further studies are needed to give chance to higher doses of Mn to show up what they are going to do .

It is hoped that the data presented here , will help to establish a scientific basis for the development of measures to minimize health hazards reluted to the production and use of nickel and its compounds and also, aid in future participation is preventive actions against the potential health effects of environmental pollutants .

REFERENCES

1. Wong CK. and Wong PK. ; Life table evaluation of the effects of cadmium exposure on the fresh water Cladoceran , Moina macrocopa . **Bull Environ Contam Toxicol** . 44: 135-141 (1990).
2. Sunderman Jr, F.W. ; Nickel in : Clarkson T.W. Frberg L. Nordberg G. F.; Sager P.R., eds . Biological monitoring of toxic metals . **New York. Plenum Press** (1988).
3. Sunderman, Jr. FW.; Hopfer, SM; Sweeney, KP; Marcus, AH; Most; BM. Creason, J. ; Nickel absorbtion and kinetics in human volunteers. **Proc. Soc. Exp. Biol** . Med. 191:5-11 (1989).
4. Kumars N.; Pant, SC.; Comparative effects on the sublethal poisoninig of zinc ,copper and lead on thr gonads of the Teleost Puntius conchonius Ham. **Toxicol Lett** . 23:189-194 (1984).
5. Sangalang GB. and OHalloran MJ.; Adverse effects of cadmium on Brooktrout testis and on in vitro testicular androge synthesis . **Biol Reprod** , 9:394 - 403 (1973).

ISSN 1110-5089

6. Rath RN, Sathyanesan AG ; Effect of mercuric chloride on the reproductive cycle of the Teleostean fish Channa punctatus **Bull Environ Contam Toxicol** 30:24-27 (1983)

7. Mills C.F., Interactions between elements in tissues, studies in animal models **Fed. Proc.**, 40:2138 (1981)

8. Clary J.J. Nickel chloride-induced metabolic changes in the rat and ginea pig **Toxicol Appl Pharmacol**, 31:55-65 (1975)

9. Sunderman Jr. FW.; Kinetics and biotransformation of nickel and chromium In Stern PM , Berlin A , Fletcher Jr., AC. eds. Health hazards and biological effects of welding fumes and gases . **Amesterdam , Excerpta Medica** (1986)

10. Kedar N and Kumar N., Gonadal histopathology following nickel intoxication in the Giant Gaurami Colisa fasciatus (Block and Schneider) , a fresh water tropical perch **Bull Environ Contam Toxicol**, 45: 299-304(1990)

11. Bertram PE; Hart BA.; Longevity and reproduction of Daphnia pulex (de Geer) exposed to cadmium contaminated food or water **Environ Pollut** . 19:295-306 (1979).

12. Sobti RC and Gill RK., Incidence of micronuclei and abnormalities in the head of spermatozoa caused by the salts of a heavy metal, nickel . **Cytologia** 54:2 ,249-253 (1989).

13. Matsumoto H.; Noda Y, Goto Y., Kishi J.; Nonogaki T.; Mori T.b; The effect of heavy metal ions on the in vitro development of mouse embryos: a comparison of the developmental ability between Ham's F-10 and alpha MEM. **Journal of Reproduction and Development** 39: 3, 223-238 (1993) .

14. Abraham G.E., Radioimmunoassay of steroids in biological materials. **ACTA Endocrinol** 75:7 (1974).

15. Allen R.M. and Redshaw M.R.; The use of homologous and heterologous I^{125} radioligands in the radioimmuno-assay of progesterone . **Steroids** (32), 467 (1979) .

16. Eastman G.J.; Corcoran J.M.; Ekins R.P.; Williams E.S. and Nabbarrow J.D., The radioimmunoassay of triiodothyronine and its clinical application. **J. of Clinical Pathology** 26:225 (1975).

17. Chopra S. J.; A radioimmunoassay for measurment of thyroxine in unextracted sera. **Metabolism** 34:938 (1972) .

18. Davis B. J.; Disc electrophoresis II . Methods and application of human serum. **Ann. N.Y. Acad. Sci.**, 121, 406 (1964) .

19. Carlstrom A and Johnson B.G. ; Electrophoresis immuno fixation Scand. **J. Immunology**,17 (10), 23 (1983) .

20. Gowenlock F. C., McMurray J. R. and McLauchlan D. N.; Varley's Practical Clinical Biochemistry, 6th Edition **Heinmann Medical Books , London**, p. 631 (1987).

21. Tallarida R.J. and Murray R.B.; Manual of pharmacologic calculations with computer programs (Edit by Sprinter-Verlag Inc. **New York, USA** P.51 (1981) .

22. Canice V.N. and Zahir A.S.; The vascular endothelium as a target tissue in acute cadmium toxicity **life Science** , 39 : 1403 - 1409 (1986).

23. Seaman C., Wyss S. and Pianeli S. ; The decline in egergic metabolism with aging of the erythrocyte and its relatioship to cell death. Amer. **J. Hematol**., 8:31-42 (1980) .

24. Sarkar B.; Nickel metabolism in nickel in the human environment JARC . Scientific piblications. **International Agency for Research on Cancer ,** (Lyon ,1984) . pp.367-381 (1984).

25. Whicher J.T.b; In biochemistry in Clinical Practice (Williams D.R. , Marks V.eds) p.223 . London: **William Heinemann Medical Books** (1983).

26. Sunderman F.W.; Jr.; Recent research on nickel carcinogenesis. **Environ, Health Perspect.**, 40: 131-141 (1981).

27. Underwood E.J.; Trace element in human and animal nutrition . 4th Ed. London, UK., **Academic Press . Inc.** p.545 (1977) .

28. El-Tohamy M.M. ; Ali G.A. and Ali U.A. ; Effects of high dietary zinc on reproductive system and adenohypophysis of male rabbits pathology and interrelation to Cu . **Egypt J., Comp. Pathol. and Clin. Pathol** ., 9: No 1 , 139-154 (1996).

29. Spiro T.G. ; Nucleic acid metal ion interactions . **New York. John, Wiley Son** ,599 (1980).

30. Sunderman F. W.; Jr. and McCully K. S.; Effects of magnesium compounds on carcinigenecity of nickel subsulfate in rats . **Carcinogenis** 4: 461-467 (1983).

31. Kasprzak K. S. ; Qnander R. V. and Polirier L. A.; Effects of calcium and magnesium salts on nickel subsulfite carcinogenesis in rats . **Carcinogenesis,** 6:1161-1166 (1985) .

32. National Research Council; Subcommittee on zinc: Zinc, **Baltimore, University Part Press** , pp.211-223 (1979) .

تاثير اعطاء النيكل على وظائف المبايض وبعض المستويات البيوكيميائية والاستيرويدات فى اناث الارانب

عبد المحسن محمد همام -ماجده محمد النهامى - جمال عبد الدايم على

قسم التكاثر الحيوانى والتلقيح الصناعى-المركز القومى للبحوث -الدقى -الجيزة

أشارت بعض الدراسات التى اجريت على الاسماك التى تعيش فى المياه العذبة والملوثة بعنصر الزنك وكذلك بعض الدببات الى توجد بعض الاثار السمية الضارة على الجهاز التناسلى لهذه السماك والحيوانات بالاضافة الى تعرض العاملين فى صناعة النيكل لبعض الاضرار الصحية فى الجهاز التنفسى وانخفاض مستوى المناعة . ولمعرفة المزيد عن تاثير كلوريد النيكل على التكاثر والخصوبة فى الحيوانات فقد تم تصميم هذه التجربة على اناث الارانب الغير بالغة لمعرفة الاثار السامة لعنصر النيكل على نشاط المبايض و الهرمونات الجنسية والتغيرات فى صورة الدم وتركيز بعض الاملاح فى الدم وبعض الانسجة وخاصة المبايض .

أجريت التجربة على عدد ٣٧ من اناث الارانب النيوزيلاندية الغير بالغة (العذارى) تم تقسيمهم الى خمسة مجموعات ز المجموعة الاولى الضابطة , المجموعة الثانية تم تجريعها بجرعة منخفضة من كلوريد النيكل(٢٠٠ مجم/حيوان) ، المجموعة الثالثة تم تجريعها بجرعة عالية من كلوريد النيكل(١٠٠٠ مجم/حيوان) ، المجموعة الرابعة تم تجريعها بجرعة منخفضة من النيكل(٢٠٠ مجم) وسلفات المنجنيز(٥٠ مجم/حيوان) . تم التجريع على مدار ٨ اسابيع متصلة بواقع ٥ مرات كل اسبوع . بينما المجموعة الخامسة لم تعطى اية معاملات او هرمونات الجونادوتروفين واعتبرت كمجموعة ضابطة ثانية .تم حقن كل المجموعات ما عدا الخامسة بجرعة مقدارها ٢٠٠ وحدة من هرمون الجونادوتروفين (PMSG) ثم اعقبها بـ ٤٨ ساعة حقن هرمون الجونادوتروفين المشيمى (HCG)بجرعة مقدارها ٢٠٠ وحده تم ذبح الحيوانات بعد اخر حقن بـ ٤٨ ساعة . تم تجميع الدم وفصل كل من البلازما والسيرم وتجميع المبايض فى محلول فسيولوجى لفحصها لتقدير عدد الحويصلات المتكونة (Follicles) والاجسام الحمراء (C.H.) والاجسام الصفراء (C.L.) فى كل مبيض . تم تقطيع المبايض وهضمها لتقدير مستوى هرمونات الاستراديول والبروجستيرون فى البلازما وانسجة المبيض بينما تم قياس مستوى الثراى ايودو ثيرونين(T3) والثيروكسين(T4) فى البلازما .كما تم بعملية الفصل الكهربائى تعيين الانواع المختلفة للبروتينات فى الدم . وتم تقدير تركيز مستويات النيكل والمنجنيز والزنك والنحاس فى البلازما وانسجة المبيض باستخدام امتصاص الطيف الذرى .أيضا تم فحص مكونات الدم وتقدير الخلايا الحمراء والبيضاء والهيموجلوبين و MCT , MCV .

أشارت النتائج ان حقن هرمون الجونادوتروفين (PMSG) ادى الى زيادة فى نشاط المبايض بصورة واضحة عن الحيوانات الغير محقونة وزيادة فى اعداد الحويصلات والاستجابة الكلية للمبايض للعلاج بالهرمونات لزيادة نشاط المبايض بينما اشارت النتائج الى ان التجريع بكلوريد النيكل بجرعة منخفضة ادى الى تثبيط لنشاط المبايض حيث انخفض معنويا عدد الحويصلات المتكونة (١٨,٠٨ ± ١,٧٧ حويصلة/مبيض) والاجسام الصفراء (٠,٤٨ ± ٠,٠٢٧) والاستجابة الكلية للمبايض(٣١,٥ ± ٠٧٥) . وبنفس الدرجة كان للجرعات الاخرى تاثير مثبط على نشاط المبايض والاجسام المتكونة فى المبيض ولم تشير النتائج ان هناك تحسنا يذكر فى نشاط المبايض بعد اعطاء المنجنيز مع النيكل . وبتقدير مستوى هرمون الاستراديول وجد ارتفاع معنوى فى كل من البلازما (٢٨٠,٥٧ ± ٦٣,٦٩ بيكوجرام /مللى لتر) وفى انسجة المبيض (٤٦٩٢,٠٩ ± ٤٤٣,٣ بيكوجرام /جرام من وزن المبيض) . بعد اعطاء جرعات منخفضة من النيكل . ووجد ايضا ان مستوى البروجستيرون قد انخفض معنويا فى البلازما فى كل المجموعات التى تم معاملتها وايضا فى انسجة المبيض فى المجموعات الثالثة والرابعة فقط مقارنة بالمجموعة الضابطة . بينما سجل الثيروكسين (T4) انخفاضا معنويا فى المجموعة الثالثة فقط . وايضا ارتفع مستوى الجلوبيولين (جاما) فى المجموعة الثانية فقط . وبفحص صورة الدم وجد ان خلايا الدم الحمراء قد تاثرت بالانخفاض فى المجموعة الثانية . بينما ارتفع عدد الخلايا البيضاء بعد اعطاء النيكل .

وبالنظر الى تركيز عناصر النيكل والمنجنيز والزنك والنحاس فى البلازما والدم بعد المعاملات المختلفة وجد ان هناك ارتفاع معنوى فى مستوى النيكل والمنجنيز بينما انخفض الزنك والنحاس بصورة كبيرة فى كل من البلازما وانسجة المبايض .