Synopsis of the Thesis on

Effect of simultaneous exposure of lead and cadmium on ovarian function in rats

Submitted to

Maharaja Sayajirao University of Baroda

For the degree of Ph.D.
(Doctor of Philosophy) in Biochemistry

By

Nampoothiri Laxmipriya Pravin

(CSIR AWARD # 9/114(103) /98/ EMR-I)

Department of Biochemistry
Faculty of Science
M.S.University of Baroda
Vadodara (Gujarat) 390002- India.

Introduction:

The problem of exposure to heavy metals like lead (Pb) and cadmium (Cd) in developed and less developed countries, and their biological effects has been well documented in recent years Metal exposure of the general population occurs chiefly through the food chain, cigarette smoke and automobiles Several reports show that lead and cadmium have serious effects on number of body functions including heme biosynthesis, energy metabolism, nervous system, behavior and coordination, kidney functions and also on reproductive system (ATSDR 1993). These effects are related to the accumulation of metals in various organs including Hypothalamic-Pituitary-Gonadal axis [HPG axis] (Ronis et al. 1996, Paksy et al 2001, Paksy et al. 1990, Vagra and Paksy 1991) This accumulation has led to disturbances in function along the HPG axis leading to ovarian malfunctioning.

Lead and cadmium are known to accumulate in the ovary (Paksy et al. 2001; Paksy et al. 1990) and gets associated with several reproductive dysfunctioning like disrupted estrus cycle, sterility and persistent estrus (Sabatelli et al. 1995; Paksy et al. 1996). Reports have indicated both metal salts cause necrosis, development of ovarian follicular cysts with the reduction in the number of corpora lutea (Rehm and Waakles 1988). Estrus cycle is under the control of pituitary hormones. Gonadotropins are released from the pituitary and binds to their receptors present on the ovary, to activate the cascade of steroidogenesis. Lead and cadmium are known to affect ovarian steroidogenesis leading to decreased production of progesterone and estrogen (Wiebe et al. 1988; Piasek and Laskey 1994).

Ovarian steroids are produced by activation of steroidogenic pathway, by the binding of Luteinising Hormone (LH) and Follicle Stimulating hormone (FSH) on to their receptors present on the ovary. There are few reports suggesting a variation in LH levels on lead exposure (Ronis et al 1996 ; Klein et al. 1994). High levels of lead are also found to result in abolition of FSH surge in rats (Petrusz et al. 1979) Cadmium exposure caused a marked decrease in preovulatory FSH, LH release (Paksy et al. 1989). Wiebe et al. (1988) showed that ovarian FSH and LH binding is decreased after lead exposure.

However, no study has been so far to understand the effect of cadmium on gonadotropin binding.

Animal studies have shown the influence of sex and age on metal toxicokinetics and toxicity in rats It was found that young, pregnant and lactating females have higher absorption of metals from the GIT and subsequent higher retention than adults. Several reports have clearly shown that lead exposure is related to impairment in progression of pregnancy (abortions and miscarriages). Various disorders of embryo/ fetus (retarded growth, malformations, increased neonatal mortality and morbidity, behavaroural teratogenicity) have also been reported. Both animal and human studies indicate that lead is readily crosses the placenta and increases lead concentration in the neonate compared to maternal blood. Acute cadmium exposure causes an alteration in maternal-embryonic relationship (Carmichael et al. 1982). Parental administration of Cd in rodents during gestation induces various teratogenic effects, which are dose dependent and species strain- specific (Gale and Layton 1980; Barr 1973). The fetoplacental unit is considered as a target for Cd toxicity during the third trimester of gestation in rodents (Levin et al. 1987). The placenta rapidly accumulates Cd after a single oral or parental administration with minimal fetal uptake. Cd exposure during gestation can produce placental hemorrhages, necrosis with high incidence of fetal death (Levin et al. 1987)

Both non-pregnant and pregnant physiological status of the animal is controlled at the cellular level. One of major component of the basic functional unit of the ovary is the granulosa cell. These cells are surrounding oocyte and committed to undergo proliferation, steroidogenic and morphological differentiation This differentiation is in response to endocrine and a paracrine stimulus, which plays a key role in the functional maturation of the entire follicle, resumed oogenesis and ovulation. Lead and cadmium accumulate in ovarian cells (Paksy et al. 2001; Paksy et al. 1990) and can change the morphology of the granulosa cell (Paksy et al. 1997; Paksy et al. 2001) which in turn would also affect the functioning of the ovary Reports have shown that lead and cadmium exposure to cultured granulosa cells caused decrease in progesterone level (Paksy et al 2001; Paksy et al. 1997)

Available data in literature suggests that there is difference in sensitivity towards metals due to difference in species, sex and physiological status and dose of toxicants. Moreover, most of studies are dealt with single metal exposure But, in reality, population is exposed simultaneously to more than one heavy metal toxicant at a time at low levels. Net effect of such an exposure would contribute to additive, antagonistic or synergistic effect. In view of this, our lab have shown that combined exposure of lead and cadmium caused a marked inhibition in activity of δ- Amino Levulinic Acid Dehydratase (δ-ALAD), marker for lead and cadmium toxicity (Gupta et al 1994). Considering all the above reports, it would be of great interest to study the effect of lead and cadmium alone and in combination on ovarian function in rats.

Objectives:

- To study the dose dependent effect of lead and cadmium in isolation and combination on ovarian function in both proestrous and estrous stages of estrus cycle.
- To study the effect of lead and cadmium in isolation and combination on reproductive performance and on biochemical parameters of placenta and ovary.
- To study the effect of lead and cadmium at cellular level using granulosa cell as the model.
- To understand the mechanism of lead and cadmium toxicity, in combination and in isolation.

❖ **Dose dependent effect of both lead and cadmium on ovarian function.**

Adult female virgin Charles foster rats weighing around 180-200g were maintained with food and water ad libitum. They were fed with pellet diet and kept under 12-hour dark and light conditions The females with regular estrous cyclicity were only chosen for the study.

Rats were divided into four groups: Group 1 received Sodium acetate (Na Ac-control), Group 2 received lead acetate (Pb Ac), group 3 received cadmium acetate(Cd Ac) and group 4 received lead acetate and cadmium acetate in combination (Pb Ac + Cd Ac). They were treated intraperitionally for 15 days daily with different doses 0.025mg/kg b.wt, 0 05 mg/kg.b.wt and 0 1 mg/kg b wt Division of regime followed for each dose and for both proestrous and estrous stages of estrous cycle. Blood was collected and animals in the respective stages were sacrificed after the treatment ovaries and uteri were removed. Dose dependent accumulation of lead and cadmium were seen in both blood and ovaries. Ovarian weight was significantly decreased in cadmium treated animals compared to other treated groups The accumulation of metal salts affected the ovarian structure and uterine structure in a dose dependent manner. Ovarian 3β Hydroxy Steroid Dehydrogenase (3β HSDH) and 17β Hydroxy Steroid Dehydrogenase (17β HSDH), key enzymes were also affected in dose dependent manner from 0.025 mg/kg b.wt to 0.05 mg/kg.b.wt without any further increase in inhibition at 0.1 mg/ kg.b.wt. Pretreatment of SH groups protectants followed by in vitro metal exposure caused an ameriolaration in enzyme activities. Uterine 3β HSDH and 17β HSDH are currently studied. Toxicity parameters (Hemoglobin, Serum Glutamate Pyruvate (SGPT), Alkaline Phosphatase (ALP) and Serum Creatinine) did not significantly change in the all metal treated groups. Cd treated animals showed maximum effect while animals receiving combined treatment showed intermediate results compared to control in all parameters studied in both proes rous and estrous stages of estrus cycle

No change in estrus cyclicity occurred, although biochemical parameters were affected. Cadmium treated animals showed maximum effect while animals receiving combined treatment showed intermediate results. From the above objective, dose chosen for further studies was **0.05 mg/kg.b.wt** *as this dose showed minimum inhibition in most parameters of Hypothalamic-Hypophyseal-Gonadal-Axis.*

❖ **Effect of lead and cadmium on reproductive performance and placenta**

Adult female rats weighing around 180-200 g with regular estrus cyclicity was chosen for the study The animals were treated subcutaneously with NaAc, PbAc, and CdAc alone and in combination of both PbAc and CdAc for 5 days as preexposure and were allowed to mate and vaginal smear was checked Presence of sperm in smear was considered as positive indicator of pregnancy For implantation experiments, rats were sacrificed on Day 9 of gestation and uteri were excised out and uterine Cathepsin D, uterine Alkaline Phosphatase were estimated , as they are important enzymes during implantation estimations were done While other rats were treated till day 19 of pregnancy Rats were sacrificed and ovary, placenta was removed and estimations were done Significant changes in the activities of both implantation enzymes were observed in all metal treated groups.

Reproductive performance was not altered in any of metal treated groups. There is significant accumulation of lead and cadmium in blood, ovaries and placenta. The combined treated group showed lesser concentration relative to individual treated groups. Biochemical parameters of placenta like protein, DNA, RNA were significantly decreased in lead treated where as cholesterol, total lipid, glycogen showed maximum decrease in cadmium treated group. Cadmium treated animals showed maximum displacement of zinc from metallothionein protein.

Placental and ovarian hormonal milieu maintain the pregnancy Both placental and ovarian key steroidogenic enzymes- 3β HSDH and 17 βHSDH activities were significantly affected in all metal treated groups. Estrogen and progesterone estimation is under investigation

Placenta and ovaries of Cd treated group showed increase in lipid peroxides, with increase activities of SOD, Catalase and decrease in reduced glutathione content in both tissues Animals exposed to combined treatment showed intermediate effects

while lead treated animals showed minimum change compared to control Toxicity parameters – ALP, SGPT and serum creatinine did not show any significant change.

The study signifies that the all biochemical parameters are altered in both ovary and placenta without any change in reproductive performance.

❖ **Effect of lead and cadmium at the cellular level using granulosa cells as the model.**

Major components of basic functional unit of ovarian follicle are the granulosa cells. These cells create and control the hormonal milieu for oocyte maturation, playing a crucial role in preconception and reproductive outcome. Therefore, all further experiments were performed in granulosa cells of ovary in non-pregnant rats, to understand the toxic effect at the cellular level.

Adult female Charles foster rats weighing 180- 200 g, with regular estrus cyclicity were chosen for the study. Pattern of treatment regime was similar to that used in non-pregnant rats, which was followed for 15 days daily at a dose of 0.05 mg/ kg. b.wt. Following the treatment, animals in the proestrus stage were sacrificed and ovaries were excised out. Granulosa cells from ovaries were isolated and gonadotropin receptor binding and key steroidogenic enzymes were estimated.

Binding of gonadotropins to their receptors triggers steroidogenesis. Cadmium treated animals shows maximum decrease in binding of r-Luteinising Hormone (r-LH) and r- Follicle Stimulating Hormone (r-FSH) compared to control. Animals which received combined treatment showed intermediate values compared to controls Steroid hormones-estrogen and progesterone also showed similar pattern of change in various treatment groups." In vitro" experiments were also performed in normal granulosa cells by exposing them to different metal salts. Again, cadmium treated animals showed a maximum decrease in binding while cells exposed to combined treatment showed intermediate values. Zinc pretreatment showed

amelioration in binding whereas pretreatment with various –SH group protectants caused in increase in activity of 17β HSDH.

The above study signifies that decreased gonadotropin binding as well as direct effect of metal salts on enzyme inhibition, which leads to decrease in hormone profile.

❖ **Mechanism of lead and cadmium in isolation and in combination at cellular level.**

To elucidate the mechanism of lead and cadmium treatment at cellular level, various membrane parameters were assessed.

Scheme of division of rats and treatment regime was same as earlier objective. After 15 days of treatment, those rats in late diestrus received 75 I.U. of hCG, to increase the yield of the cells. Later, sacrificed after 24 hours at proestrous stage and cells were isolated and membrane fluidity, membrane Na+ K+ ATPase activity was estimated along with the cellular cholesterol and phospholipid content. All parameters were significantly decreased except an increase in fluidity was seen in all metal treated groups.

Since metals are known to generate free radicals, granulosa cells from control and metal treated animals were assessed for oxidative stress parameters. Cells of cadmium treated animals showed higher lipid peroxidation with higher superoxide dismutase activity and catalase activity and a maximum decrease in reduced glutathione content compared to control All parameters were intermediate in cells of combined treated animals while cells of lead treated animals showed minimum change.

Similar parameters were assessed in "in vitro" experiments Most of the parameters did not show any significant change except in Na+K+ ATPase activity, lipid peroxidation and reduced glutathione levels.

Thus, biochemical changes caused by heavy metals are due to production of free radicals leading to alteration in membrane structure and antioxidant system.

References:

Agency for Toxic Substances and Disease Registry (**ASTDR**). (1993) Lead, Cadmium. Toxicological profiles for lead and cadmium: Update.TP-92/06,12. Atlanta GO: US Department of health and human services, Public Health Services, ATSDR.

Barr M (1973) The teratogenecity of cadmium chloride in two stocks of Wistar rats. *Teratology*, 7, 237-242

Carmichael NG, Backhouse BC, Winder C, Lewis P. (1982) Teratogenecity, toxicity and perinatal effects of cadmium. *Human Toxicol.*, **1**, 159-186.

Gale TF, Layton WM. (1980) The susceptibility of inbred stains of hamsters to cadmium induced embryotoxicity. *Teratology*, **21**, 181-186.

Gupta S, Bhosale S, Pandya K (1994) Effect of simultaneous low level exposure of Pb and Cd on δ-ALAD and acetylcholinesterase activity in rats. *Indian J. of Exp. Biol.*, **32,** 819-821.

Klein D, Wan YJ, Kamyab S, Okuda H, Sokol RZ. (1994) Effects of Toxic levels on lead on gene regulation in the male axis: Increase in messenger RNA and intracellular stores of gonadotrophs within the central nervous system. *Biol. Reprod.* , **50**, 802-811.

Levin AA, Klipper RW, Miller RK.(1987). Fetal toxicity of cadmium chloride: pharmokinetics in pregnant Wistar rat. *Teratology*, **36**, 163-170.

Paksy K, Varga B, Horvath E, Tatrai E, Ungvary G. (1989) Acute effects of cadmium on preovulatory serum FSH, LH and Prolactin levels and on ovulation and ovarian hormone secretion in estrous rats *Reprod. Toxicol* , **3**, 241-247.

Paksy K, Naray M, Varga B, Kiss I, Folly G, Ungvary G. (1990) Uptake and distribution of Cd in ovaries, adrenals and pituitary in pseudopregnant rats: Effects of Cd on progesterone serum levels *Envt. Res.*, **51**, 83-90

Paksy K, Vagra B, Lazar P. (1996) Effects of Cd on female fertility, pregnancy and postnatal development in rats. *Acta Physiologica Hungaraia*, **84**, 119-130.

Paksy K, Rajczy K, Forgacs Z, Lazar P, Bernard A, Gati I, Kaali GS (1997) Effect of Cd on morphology and steroidogenesis of cultured granulosa cells. *J. Appl. Toxicol.* **17**, 321-327

Paksy K, Gati I, Naray M, Rajczy K. (2001) Lead accumulation in human ovarian follicular fluid and in vitro effect of lead on progesterone production by cultured human ovarian granulosa cells. *J. Toxicol. Envt Health, Part A,* **62**, 359-366.

Piasek M, Laskey LW (1994) Acute Cadmium exposure and ovarian steroidogenesis in cycling and pregnant rats. *Reprod. Toxicol* , **8**, 497-507.

Pertusz P, Weaver CM, Grant LD, Mushak P, Krigamn MR. (1979) Lead poisoning and reproduction Effects on pituitary and serum gonadotropins in neonatal rats *Envt. Res* , **19**, 383-391.

Rehm S, Waakles MP (1988) Cadmium induced ovarian toxicity in Hamsters, mice's and rats *Fundament Appl. Toxicol.* **10**, 635-47.

Ronis M.J.J., Badger T.M., Shema S.J., Roberson P.K, Shaikh F.(1996) Reproductive Toxicity and growth effects in rats exposed to lead at different periods during development. *Toxicol. Appl. Pharmacol.* **136**, 361-371.

Sabatelli G, Sacco A, Castellino P. (1995) Lead and reproductive system In: Castellino N, Castellino P, Sannolo N; eds. Inorganic lead exposure. Metabolism and intoxication Baco Raton FL: Lewis Publishers/ CRC Press, Inc. 423-436.

Wiebe JP, Barr KJ, Buckingham KD. (1988) Effect of prenatal and neonatal exposure to lead on gonadotropin receptors and steroidogenesis in rat ovaries. *J. Toxicol. Envt. Health*, **24**, 461-476.

Varga B, Paksy K (1991) Toxic effects of Cd on LHRH induced LH release and ovulation in rats. *Reprod. Toxicol.*, **5**, 199-203.

Research Papers communicated:

- **Title: "Dose dependent effect on simultaneous exposure of lead and cadmium on ovarian function in proestrous rats."**

  Authors: **Laxmipriya P.N** , Anilkumar Pillai and Sarita Gupta

  Journal: **Bulletin of Environmental Contamination and Toxicology.**

- **Title: "Effect of simultaneous exposure of lead and cadmium on gonadotropin receptors of granulosa cells: an " *in vitro* " study."**

  Authors: **Laxmipriya P.N** , Anilkumar Pillai and Sarita Gupta

  Journal: **Toxicology**

- **Title: Combined exposure of lead and cadmium on hepatic antioxidant system and lipid peroxidation**

  Authors Anil Pillai, **Laxmipriya** and Gupta S

  Journal: **Journal of Trace Elements in Medicine and Biology**

Research Papers Published/ Accepted:

- **Title: Simultaneous exposure of lead and cadmium on granulosa cells, progesterone and luteinising hormone in proestrous rats.**

  Authors: Sarita Gupta, **Laxmipriya** and Vishal Gohil.

  Journal: **Advances in Pharmacology and Toxicology, 3(2), 87-93, 2002 (In Press)**

- **Title: Effect of low level exposure of lead and cadmium on hepatic estradiol metabolism in female rats.**

  Authors: Pillai A, **Laxmipriya**, Rawal A, Gupta S

  Journal: **Indian Journal of Experimental Biology, 40, 807-811, 2002.**

- **Title: Effects of combined exposure of lead and cadmium on pituitary membrane of female rats.**

  Authors: Pillai A, **Laxmipriya** and Gupta S

  Journal: **Archives of Toxicology (in press)**

- **Title: Effects of combined exposure of lead and cadmium on hypothalamic-pituitary axis function in proestrous rats.**

  Authors: Pillai A, **Laxmipriya** and Gupta S

  Journal. **Food and Chemical Toxicology (in press)**

(Laxmipriya P. Nampoothiri)

(GUIDE) (OFFG. HEAD) (DEAN)