Chapter –IV

EFFECT OF LEAD AND CADMIUM ON REPRODUCTIVE PERFORMANCE AND PLACENTA

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Pregnancy is a main physiological aspect of life. Ovum after fertilization undergoes implantation that involves the breakdown of complex proteins and polysacchharides, which are mediated by lysosomal enzymes namely Cathepsin –D and alkaline phosphatase (Moulton, 1974). Following the implantation, there is development of a specialized structure called placenta. Placenta maintains maternal – embryonic relationship. Both human and animal data suggests that lead is easily permeable through placenta, which causes high lead concentrations in the fetal blood when compared to maternal blood (Sabatelli et al., 1995). Permeability of placenta to lead causes an alteration in several essential elements homeostasis (Blanusa et al., 1989; Miller et al., 1990). In contrast to lead, cadmium is known to get accumulated in the placenta, which therefore acts as an important, but not complete barrier to protect the fetus from toxic effects of cadmium, as reported in rodents (Webster, 1979) and in humans (Korpela et al., 1986). Parental administration of cadmium in rodents during gestation induces various teratogenic effects, which are dose dependent and species and strain specific (Barr, 1973; Gale and Layton, 1980). Cadmium exposure during gestation is known to cause placental necrosis and hemorrhages, with increased rate of fetal death (Samarawickrama and Webb, 1979; Samarawickrama and Webb, 1981; Levin et al., 1987).

It is clear from the earlier chapter that lead and cadmium exposure both in isolation and in combination causes an alteration in the structure of ovary and uterus along with significant inhibition in steroid biosynthesis in non-pregnant rats. Hence, it would be of great interest to study the effect of lead and cadmium either alone or in

would be of great interest to study the effect of lead and cadmium either alone or in combination during implantation and at term during gestation in rats. The present study was undertaken to analyze the effect of these heavy metals on reproductive performance, implantation, placental protein profile, ovarian and placental steroidogenesis, along with distribution of metals.

Experimental design

Adult virgin female rats (200-220 g) were divided into four groups each consisting of 12-13 animals. First group received subcutaneous injections of sodium acetate (control), second and third groups were treated with lead acetate and cadmium acetate respectively. The fourth group of animals were treated with combined dose of lead acetate and cadmium acetate at a dose of 0.05 mg/ kg .body wt/ day subcutaneously. On the fifth day of treatment, those animals that were in late diestrus to early proestrous stage of estrus cycle were allowed to mate with males. Presence of thick cornified smear, along with sperm on the next day confirmed the pregnancy. Five to six animals from each group those confirmed for mating and taken as day 1 of pregnancy. They were sacrificed on fifth day of gestation and assessed for implantation enzymes – Alkaline Phosphatase (Bowers & Mc Comb, 1975) and Cathepsin-D (Anson, 1937). Other animals were continuously exposed to metals till day 19 of gestation. At the end of the treatment, blood samples were collected and centrifuged at 3000 rpm for 15 min. Serum was separated and stored at –20 °C, till sex hormones- estradiol and progesterone were estimated using Coat-A kit (DPC, USA). Later, animals were sacrificed, ovaries and placenta were removed and processed for estimation of steroidogenic enzymes- 3β Hydroxy Steroid Dehydrogenase (3β HSDH) and 17β Hydroxy Steroid Dehydrogenase (17β HSDH) (Shivanandappa and Venkatesh, 1997). Placental metallothionein (Bayne et al., 1985)

was estimated in microsomal fraction. Biochemical parameters such as DNA (Burton, 1968), RNA (Schneider, 1957), total lipid (Folch et al., 1957), cholesterol (Leffler and Mc Dougald, 1963), glycogen (Seifter et al., 1950) and protein content (Lowry et al., 1953) were estimated in placenta. Electrophoretic profile of placental proteins was observed on 10% SDS-PAGE (Lamelli, 1970). Toxicity parameters such as Serum GPT (Reitman and Frankel, 1957), ALP (Bowers & Mc Comb, 1975) and creatinine levels (Bonsnes and Taussky, 1945) were analyzed to check the toxicity of administered dose. Lead and cadmium content in blood, ovaries and placenta of pregnant animals were estimated using GBC902 Atomic Absorption Spectrophotometer.

Results

Lead and cadmium either alone or in combination had no significant effect on reproductive performance (Table1). There was no change in fertility rate, litter size and litter weights in all metal treated groups. Body weights, ovarian and placental weights demonstrated no significant change but the implantation enzymes- Cathepsin-D and alkaline phosphatase activities were altered on metal exposure (Figure 1 and 2).

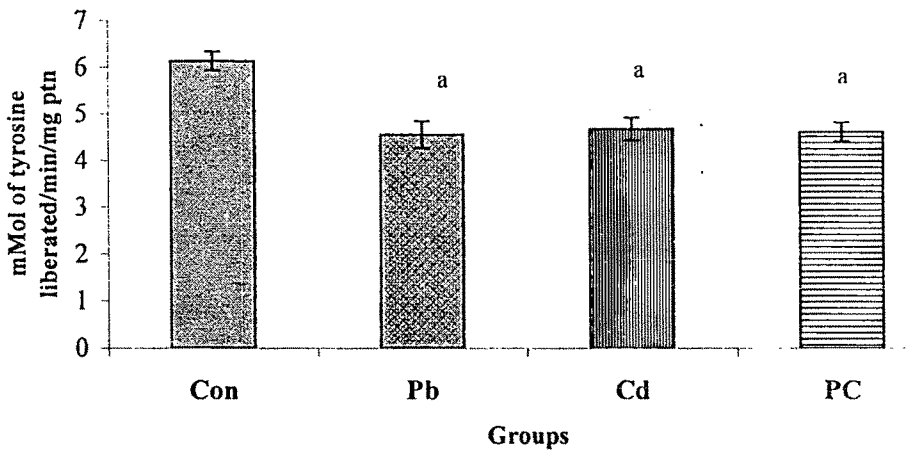
The concentrations of lead and cadmium in the blood, placenta and ovary are shown in Tables (2 and 3). Table 4 shows the zinc concentration in metallothionein fraction of placenta. Cadmium and combined treated animals showed maximum displacement of zinc.

Table 5 shows the effect of lead and cadmium either alone or in combination on biochemical parameters of placenta. Protein and RNA content showed maximum change

Table 1: Effect of lead and cadmium either alone and in combination on reproductive performance of rats.

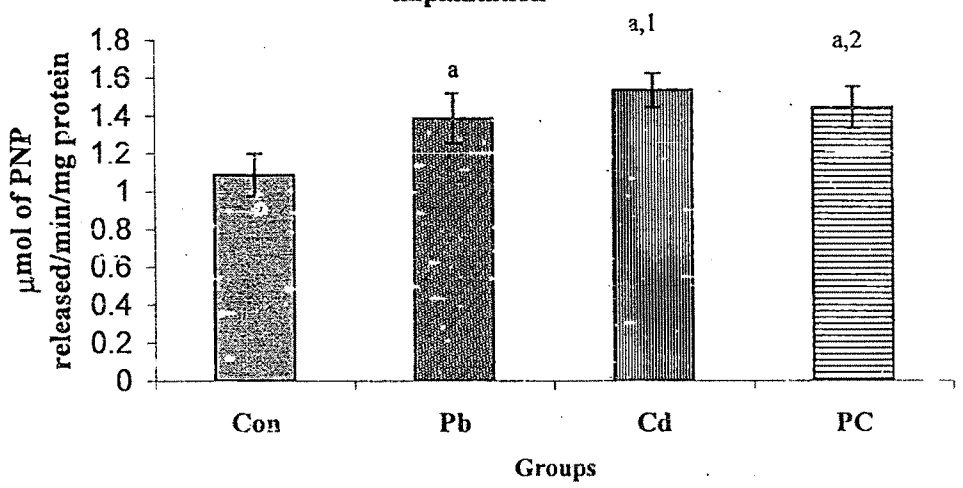
Parameters	Control	Lead	Cadmium	Lead +Cadmium
Number of animals kept for mating	14	14	14	14
Number of animals conceived	12	11	12	12
Body weight at conception (g)	192 ± 5.11	202 ± 8.47	188.6 ± 8.1	185 ± 7.92
Body weight at Gestation day 19 (g)	274.5 ± 7.97	264.5 ± 9.93	251.6 ± 5.7	258. 7 ± 6.35
Rel wt. gain (%)	8.6 ± 1.21	7.62 ± 0.89	11.76 ±1.56	17 ±2.6
Litter Size	8.0 ± 0.5	10.77 ± 0.52	8.78 ± 0.5	8.3 ± 0.54
No. Of Dead/ resorbed	1 ±0.005	2 ± 0.10	3 ±0.5	2 ±0.2
Total litter weight (g)	63 ±9.75	55.83 ± 15.94	59 ± 10.84	46 ± 8.22
Ovarian weight (mg)	229.6 ± 9.06	211.36 ± 29.04	207.3 ± 14.16	203. 1 ± 9.7
Placental weight (g)	4.5 ± 0.119	4.39 ± 0.363	4.19 ± 0.24	4.48 ± 0.18

Figure 1: Effect of lead and cadmium either alone or in combination on Cathepsin-D activity during implantation.



n=3-4 . The values are mean ±SEM.
a P<0.001 compared to control

Figure 2: Effect of lead and cadmium either alone or in combination on Alkaline Phosphatase activity during implantation



n=3-4 . The values are mean ±SEM.
a P<0.001 compared to control
1 P<0.001, 2 P<0.05 compared to lead

Table 2: Distribution pattern of lead and cadmium in blood during pregnancy.

Group	Concentration of lead (μg/ml)	Concentration of cadmium (μg/ml)
Control	0.60 ±0.083	0.433 ±0.088
Lead	2.49 ±0.272 [a]	0.450 ± 0.0707
Cadmium	0.733 ±0.0623 [1]	1.362 ± 0.0982 [a,1]
Lead + Cadmium	1.25 ±0.0957 [1]	0.875 ±0.4787 [c,2]

N=3-4. The values are mean ±SEM.
a P<0.001 c P<0.05 compared to control
1 P<0.001, 2 P<0.01 compared to lead group

Table 3: Distribution patterns of lead and cadmium in reproductive tissues during pregnancy.

Groups	Concentration of lead		Concentration of cadmium	
	Ovary (ng /mg wet wt)	placenta (μg/g wet wt)	Ovary (ng /mg wet wt)	placenta (μg/g wet wt)
Control	0.323 ± 0.0101	0.4 ±0.1034	0.415± 0.04	0.338 ±0.0221
Lead	0.989 ± 0.009 [b,**]	2.4 ±0.010 [a,***]	0.515 ±0.05	0.466 ±0.067
Cadmium	0.46 ± 0.0027	0.477 ±0.09	1.24 ±0.019 [b,2]	3.15 ± 0.0247 [a, 1]
Lead + Cadmium	0.76±0.008 [c, *]	0.804± 0.023 [c, 1,*]	0.63 ±0.096 [**]	1.28 ± 0.022 [a,1, **]

N= 3-4. The values are mean + SEM.
a P<0.001 b P<0.01, c P<0.05 compared to control
1 P<0.001, 2 P<0.01 compared to lead group
*** P<0.001, ** P<0.01, * P<0.05 compared to cadmium group

Table 4: Effect of lead and cadmium either alone or in combination on zinc level in placental metallothionein fraction.

Groups	Concentration of Zn (μg/mg protein)
Control	0.141 ±0.006
Lead	0.064 ±0.08 [a]
Cadmium	0.045± 0.011 [a,2]
Lead +Cadmium	0.051 ± 0.0132 [a, 2]

N=4-5. The values are mean ± SEM.

a $p<0.001$ compared to control group

[2] $P<0.01$ compared to lead group

Table 5: Effect of lead and cadmium either in isolation or combination on the biochemical parameters of placenta.

Group	Control	Lead	Cadmium	Lead + Cadmium
Protein (mg/g tissue)	45.74 ± 0.244	35.49 ±0.273 [a,2]	39.31 ±1.26 [b]	40.64 ± 1.83 [c]
DNA (mg/g tissue)	1.75 ±0.156	1.458 + 0.176 [a]	1.321 + 0.037 **	0.925 ± 0.085 [b] *, B
RNA (mg/g tissue)	6.66 ± 0.28	2.7 ± 0.228 [a]	3.14 ± 0.065 [a]	3.35 ± 0.133 [a]
Cholesterol (mg/g tissue)	13 ± 1.05	7.97 ± 0.198 [a, A]	6.33 ± 0.21 [a]	8.115 ± 0.209 [a, A]
Total lipid (mg/g tissue)	58.78 ±0.246	37.72 ± 0.133 [a, A]	23.32 ±0.91 [a]	47.79 ±0.688 [a, A]
Glycogen(mg/g tissue)	0.424 ± 0.02	0.223 ±0.012 [a]	0.246 ±0.005 [a]	0.236 ±0.012 [a]

N=5-7. The values are mean ±SEM.

a $p<0.001$, b $p<0.01$, c $p<0.05$ compared to control

** $p<0.001$, * $p<0.01$ compared to lead group.

A $p<0.001$, B $p<0.01$ compared to cadmium group.

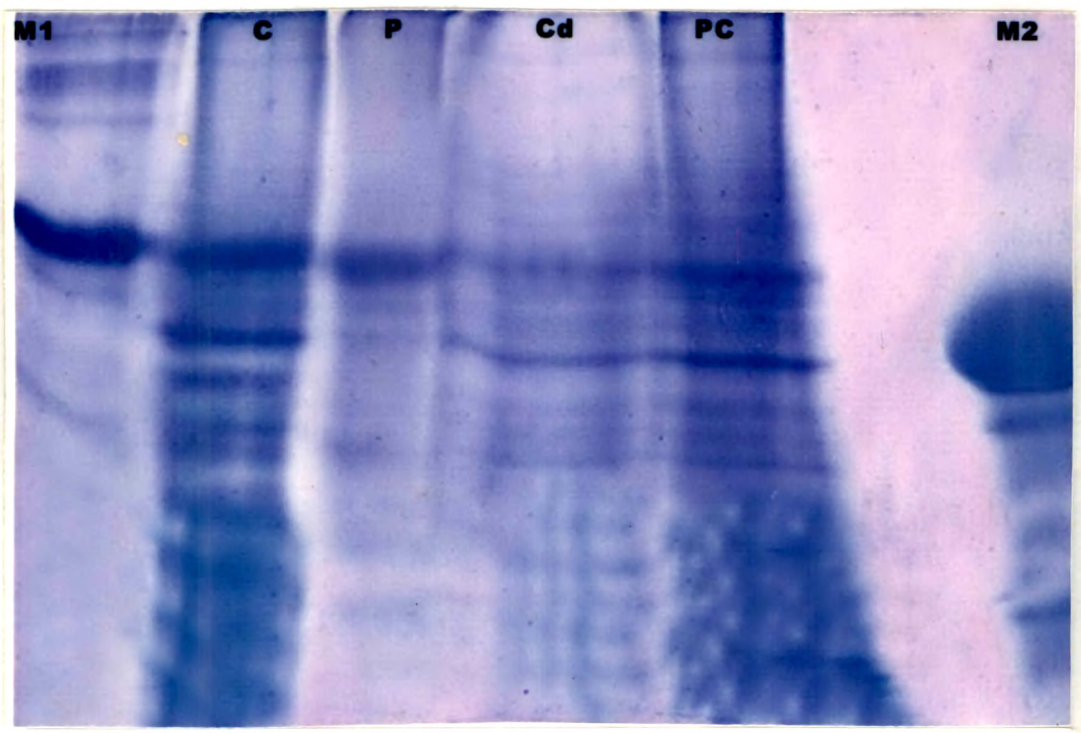
2 $p<0.05$ compared to lead + cadmium group

in placenta of lead treated animals, while placenta of cadmium treated animals showed minimum change as compared to control. Maximum decrease of DNA has been observed in combined treated animals Cholesterol and total lipids were maximally decreased in placenta of cadmium treated animals Glycogen content (approximately 50%) was decreased in all metal treated groups. In most of the parameters, the animals receiving combined treatment showed intermediate results. Electrophoretic pattern of different proteins is represented in Plate 1. The intensity of most of the bands is decreased in all metal treated groups. In case of 30 kDa protein, there is a sharp decrease in lead treated animals and 66 kDa protein expression has been decreased relatively in cadmium treated group.

Figures 3 and 4 shows the effect of lead and cadmium either alone and in combination on steroid dehydrogenases in placenta and ovary. Lead treated group showed minimal change in the key steroidogenic enzyme activity (3βHSDH & 17βHSDH). The animals receiving cadmium and combined treatment demonstrated almost similar extent of inhibition for steroidogenic enzymes, in both the reproductive organs.

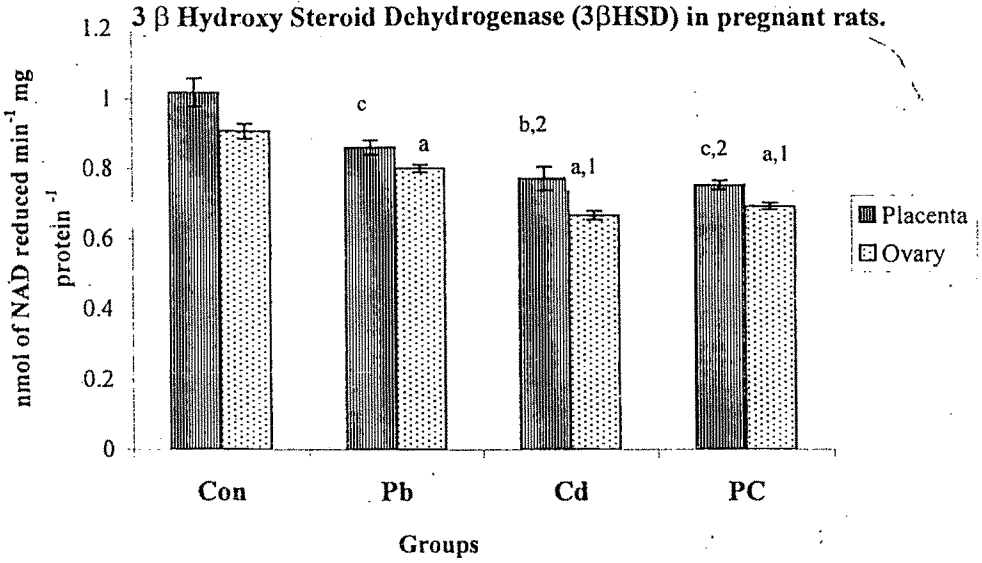
The steroids- progesterone and estradiol production was significantly affected in all metal treated groups in serum, ovary and placenta. Lead treated animals revealed a minimum change in steroid hormones as compared to control. Animals receiving cadmium and combined treatment demonstrated a maximum decrease in steroid hormone (estradiol and progesterone) production in both ovary and placenta while serum gonadal steroids levels were intermediate in combined treated group (Figures 5(a, b and c) and 6 (a, b and c).

Plate 1: Electrophoretic Protein profile of placenta after lead and cadmium exposure



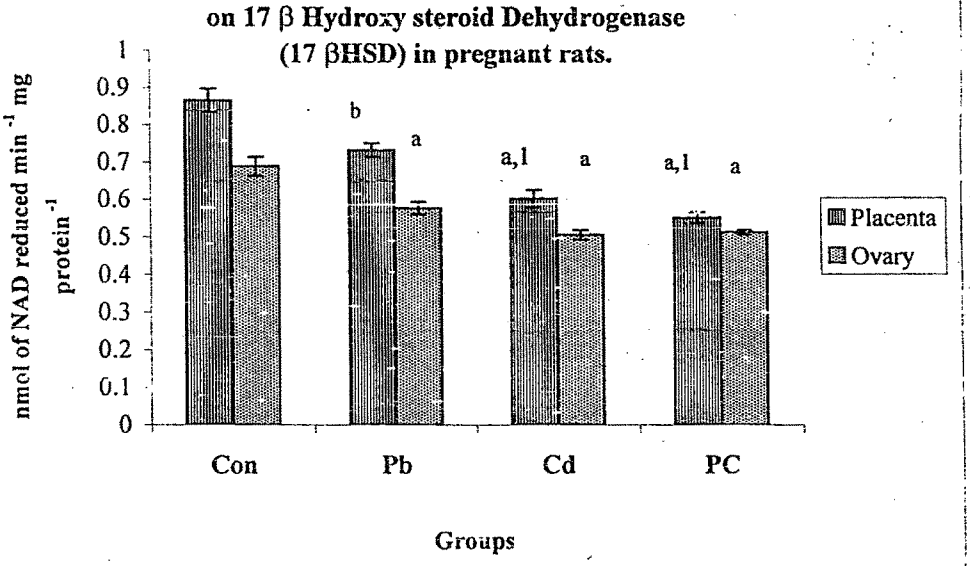
M1- 66 kDa (BSA) M2-34 kDa (pepsin) C- Control. P- Lead, Cd – Cadmium PC- lead +Cadmium

Figure 3: Effect of lead and cadmium alone and in combination on 3 β Hydroxy Steroid Dehydrogenase (3βHSD) in pregnant rats.



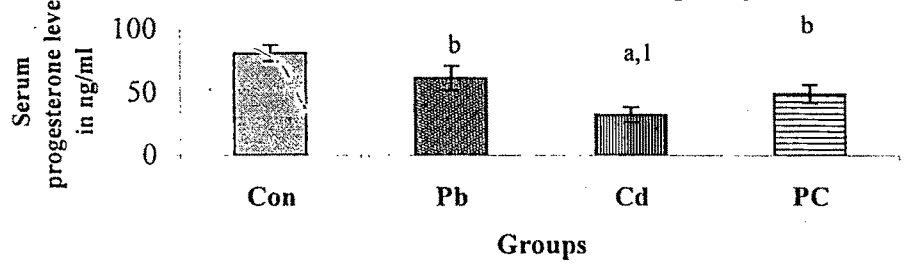
N=6-8. The values are mean ±SEM
a P<0.001, b P<0.002, c P<0.01 compared to control
1 P<0.001,2 P<0.05 compared to lead group.

Figure 4: Effect of lead and cadmium alone and in combination on 17 β Hydroxy steroid Dehydrogenase (17 βHSD) in pregnant rats.



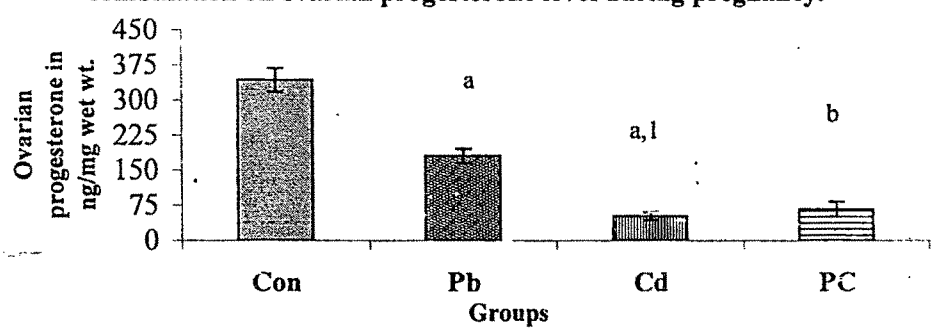
N=6-8. The values are mean ±SEM
a P<0.001, b P<0.002, c P<0.01 compared to control
1 P<0.001 compared to lead group.

Figure 5a: Effect of lead and cadmium either alone or in combination on serum progesterone levels in pregnancy



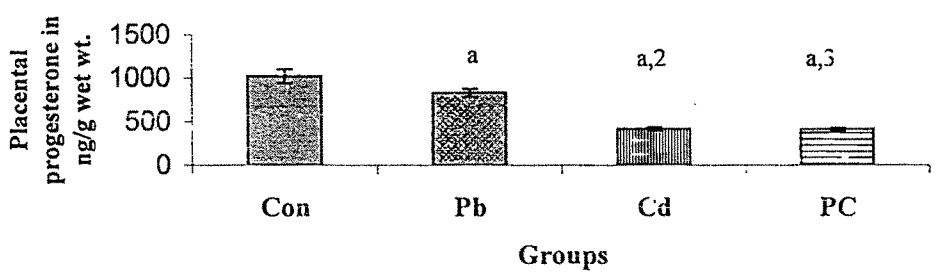
n=4-5. The values are mean + SEM.
a p<0.001,b P<0.01 compared to control group
1 P<0.01 compared to lead group

Figure 5b: Effect of lead and cadmium either alone or in combination on ovarian progesterone level during pregnancy.



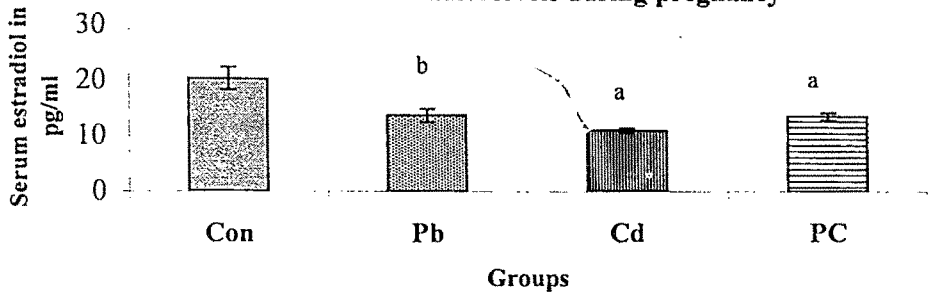
n=4-5. The values are mean + SEM.
a p<0.001,b P<0.01 compared to control group
1 P<0.01 compared to lead group

Figure 5c: Effect of lead and cadmium either alone or in combination on placental progesterone levels



n=4-5. The values are mean + SEM.
a p<0.001,b P<0.01 compared to control group
2 P<0.01, 3 p<0.05 compared to lead group

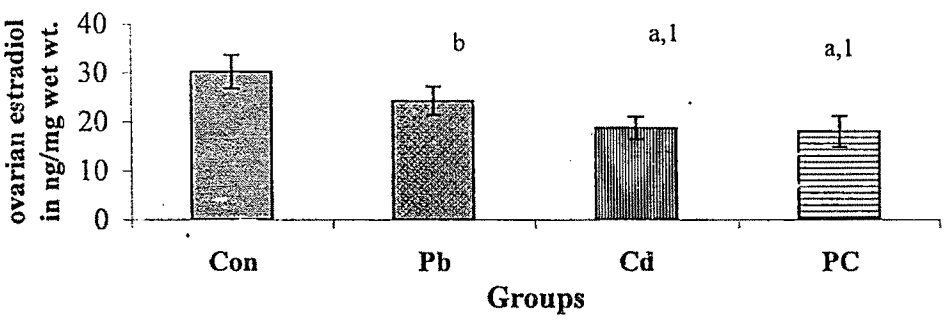
Figure 6a: Effect of lead and cadmium either alone or in combination on serum estradiol levels during pregnancy



n=4-5. The values are mean ± SEM.

a $p<0.001$, b $P<0.01$ compared to control group

Figure 6b: Effect of lead and cadmium either alone or in combination on ovarian estradiol level

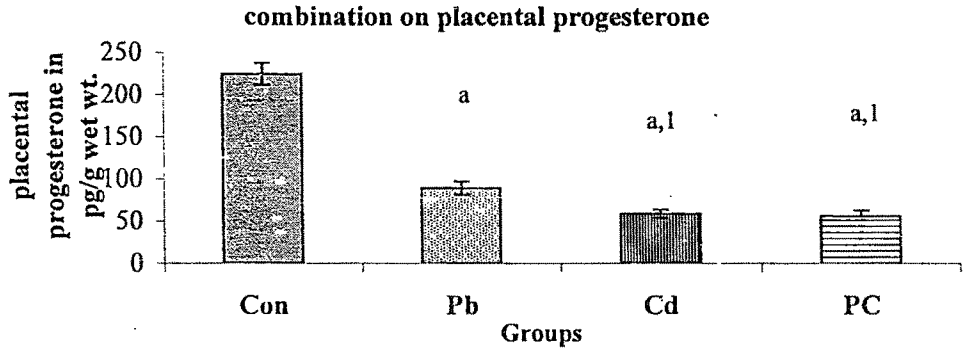


n=4-5. The values are mean ± SEM.

a $p<0.001$, b $p<0.01$ compared to control group

1 $p<0.001$ compared to lead group

Figure 6c: Effect of lead and cadmium either alone or in combination on placental progesterone



n=4-5. The values are mean ± SEM.

a $p<0.001$ compared to control group

1 $p<0.001$ compared to lead group

Table 6: Effect of lead and cadmium either alone or in combination on safety parameters

PARAMETERS	CONTROL	LEAD	CADMIUM	LEAD + CADMIUM
Serum Glutamate Pyruvate trans-aminase (SGPT) (IU/ L)	20.53 ± 1.27	20.97 ±0.924	19.93 ± 1.49	19.19 ±0.926
Serum Creatinine (mg %)	1.185 ±0.0536	1.35 ±0.0687	1.99 ± 0.113	2.85 ± 0.1087
Serum Alkaline Phosphatase (ALP) (IU/ L)	83.1 ±5.12	66.45 ±4.23	72.24 ±4.29	65.83 ± 6.25

N=6. Values are Mean ± SEM.

ALP: IU- μ moles of PNP formed/ min

SGPT: IU-μ moles of pyruvate formed/ min

SGPT, a marker of liver damage showed higher activity, while serum ALP showed decreased activity and serum creatinine levels were elevated in all treated groups. However, change in all safety parameters were within the normal range (Table 6).

Discussion

Reproductive cycle was not altered in any of the metal exposed groups. Rate of pregnancy was equally distributed in all metal-treated groups. All the parameters including fetal weight, litter size, ovarian weights and placental weights showed no change. Thus, lead and cadmium administration before conception and when continued till end of gestation could not demonstrate any toxic effect on reproductive parameters. In contrast to these observations, implantation enzymes like Cathepsin-D and Alkaline phosphatase did get affected. Increase in alkaline phosphatase activity was in accordance to previous reports (Nichollas et al., 1983; Nehru and Kaushal, 1993). Cathepsin-D activity demonstrated a decrease, may be due to the fact that lead and cadmium competes with magnesium ion to bind at its active site, thereby decreasing the activity. Inspite of changes in lysosomal enzymes, implantation sites did not seem to be affected.

Cadmium is a proven teratogenic agent in early post-implantation on day 8 (Denker, 1975), in mid pregnancy and late gestation (Parizek, 1983). It has been reported that the fetoplacental unit is considered as a target for cadmium toxicity mainly during third trimester of gestation in rodents (Levin et al., 1983). Hence, administration of these metals during late gestation period would pose a greater risk of toxicity. Adaptive mechanism like the induction of placental metallothionein would partially help in sequestering of metal ions, which would render protection against these heavy metals (Goyer and Cherian, 1992). In rat placenta, Cadmium is known to

induce rapid synthesis (Goyer and Cherian, 1992), thereby reduces the bioavailability of highly toxic free metals. Displacement of zinc in the metallothionein fraction had been demonstrated maximally in cadmium treated animals while lead has caused less displacement of zinc. Thus, observed change in combined metal exposed group could be mainly due to inhibitory effects produced by cadmium.

Progesterone and estrogen are main gonadal steroids that are required to maintain pregnancy. The steroidogenic enzymes- 3βHSDH and 17β HSDH activities were decreased in reproductive organs (i.e., ovary and placenta) in all metal treated groups. The inhibition obtained could be related to metal binding to sulfhydryl groups as discussed in the previous chapter. Inhibition of steroidogenic enzymes can be associated with decrease of steroid hormones (progesterone and estradiol) as observed in our study. Inhibition in steroid production on lead exposure could be related to decreased expression of Steroidogenic Acute Regulatory protein (StAR) (Huang et al., 1997; Liu et al., 2001) and steroid dehydrogenases (Wiebe et al., 1982). Piasek and Laskey (1994) reported that cadmium at the doses of 3 and 5 mg/kg body wt. were associated with perturbations in ovarian and placental steroidogenesis. It was recently reported that sub chronic administration during 19 days of pregnancy at a total dose of 5 mg/kg. body wt in rats caused a decreased production of placental progesterone (Piasek et al., 2002). Joliobios (1999a, b) have demonstrated a dose and time dependent bioaccumulation of cadmium in purified trophoblast cells in primary culture, associated with concomitant inhibition of progesterone synthesis. He also postulated other mechanisms by which cadmium may exert its effect through altered intracellular trafficking of cholesterol into mitochondria, activities of mitochondrial P450 scc or 3βHSD and or interference of cadmium with zinc finger motif on DNA transcription. Decreased progesterone production could be competition with other

calcium dependent pathways in trophoblast cells (Lin et al., 1997). A dose dependent decrease was also reported in P450scc and 3βHSD mRNA transcripts in trophoblasts, cocultured with cadmium chloride (Kawai et al., 2002).

Electrophoretic protein profile on comparing with the marker protein demonstrated decreased expression of 30-34 kDa and 60-66 kDa protein in lead and cadmium treated groups respectively. On the basis of placental protein profile from various reports, it seems that proteins whose expression are decreased could be either StAR (34 kDa, important protein for cholesterol transport in steroidogenesis), PP5 (36 kDa, an anti-coagulant present on the surface of syncytiotrophoblasts) or Gir protein (66 kDa, a unique placental protein, which plays a role in insulin signaling). Since, western blot analysis could not be done performed, nothing can be said conclusively. Both the metals either in isolation or in combination might be interacting with transcriptional machinery either directly or indirectly and thus causing reduction in expression as reported by several workers (Watkin et al., 2003; Pennypacker et al., 1997).

Glucose is needed to meet the energy requirements of the growing embryo. Our present study demonstrated decrease in glycogen content in all metal treated groups. Hazelhoff Roelfzema et al. (1988) reported that cadmium exposed animals have less glycogen content after gestational day 18. Mothers exposed to lead during gestation and lactation have decreased hepatic glycogen content, with increased level of glucose in the blood (Corpas et al., 1996). Our study showed a decrease in DNA, RNA and protein content in all treated groups. Such observations has also been reported earlier by various workers (Antonio et al., 1999; Corpas et al., 1996). Biomolecules like cholesterol, total lipids showed a maximum decrease in cadmium treated group than the other groups. Decrease in cholesterol content obtained in our

present study can be associated with decreased progesterone production. It has been implicated that cadmium causes a dose dependent reduction in LDL-receptor mRNA level in trophoblast cells (Joliobios et al., 1999b). Safety parameters like SGPT, ALP activities and Serum creatinine levels are getting altered, but lies within the normal range.

Pregnancy is a state of physiological stress and often get affected even though effects could not be demonstrated in non pregnant state. It is surprising to note from above results that administration of metals at a dose of 0.05 mg/kg. body wt./day from premating time till end of term could only result in sub-clinical toxicity (derangement in various biochemical parameters), without demonstrating any effect on reproductive performance (mating), implantation or pregnancy outcome. Since steroidogenic enzymes, steroid hormones, energy substrate (glucose and lipids), DNA, RNA and protein profile had been affected. It can be speculated that long time exposure may affect reproductive health of mother and their progeny.

Summary

Adult synchronized virgin females were treated subcutaneously (0.05 mg/ kg .body wt ./day) with sodium acetate (Control), lead acetate and cadmium acetate alone and in combination during gestational period , with pretreatment of 5 days prior to mating. There were no alterations in reproductive performance in all metal treated groups. Implantation enzymes- cathepsin-D and alkaline phosphatase activity were altered , but no change in frequency of implantation sites. Ovarian, placental and body weights, litter number did not show any change in metal treated groups. The key enzymes of ovarian and placental steroidogenesis (3βHSD and 17βHSD) were affected the most in cadmium and combined treated animals while lead treated animals showed minimum change compared to control group. This decrease was correlated to decrease in gonadal steroid levels both in serum and reproductive tissues. Maximum displacement of zinc bound to metallothionein was more in cadmium and combined treated rats compared to other treated groups. Biomolecules like glycogen, protein, RNA, DNA and protein content were affected in all metal treated group. Cadmium treated animals showed greater effect on biomolecules like cholesterol, lipid content compared to other groups. Toxic parameters like ALP, SGPT and creatinine were altered but were within the normal range. Biochemical effects are correlated with metals accumulated in blood, reproductive tissue like placenta and ovary.