Chapter VII
SUMMARY

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Human reproduction and development occurs in an environment contaminated by several heavy metal pollutants mainly lead and cadmium. Concern for the reproductive health of females is mounting, as they bear progeny of the future. Ovary is the crucial organ that provides viable oocytes for fertilization and toxicity could result in functional alterations in ovarian physiology, causing a profound influence on reproductive function. Most of the studies lead and cadmium as reproductive toxicants are in context with ovary, pregnancy and fetal outcomes and have dealt mainly with single metal in isolation. In reality, human population are constantly exposed to more than one metal at a time. Therefore, net result of such an exposure could be either additive, antagonistic or synergistic. Therefore, it is essential to understand the effect of simultaneous exposure of both lead and cadmium in combination on ovarian function. In the present study, the effects of lead and cadmium, either alone or in combination on ovarian function during non-pregnant and pregnant states have been evaluated. In addition, granulosa cells of ovary has been used as a cellular model and studies have been done to understand the mechanism of toxicity caused by these metals.

Adult female Charles foster rats were treated with lead and cadmium either alone or in combination in dose dependent (0.025, 0.05 and 0.1 mg/kg .body wt.) and time dependent manner (15, 30 and 45 days) and key steroidogenic enzymes (3β hydroxy steroid dehydrogenase [3β HSDH] and (17β hydroxy steroid dehydrogenase [17β HSDH], steroids were assessed in ovary and uterus. All metal treated groups showed significant inhibition of the key enzymes in dose dependent manner (from 0.025 to 0.05

mg/kg. body wt), but no significant inhibition was obtained as dose increased from 0.05 to 0.1 mg/ kg. body wt.). In time dependent studies, both key enzymes showed significant inhibition in lead and combined treated as time of treatment increased from 15 days to 30 days, but no significant change was observed as time increased from 30 days to 45 days. In case of cadmium treated group, further decrease in 3β HSDH activity was not seen as time of exposure increased, but 17β HSDH did show a change. Inhibition of the key enzymes could be related to binding of metals to sulfhydryl groups present in NAD binding domain of the enzymes (Ouig, 1998) and was partly correlated with decreased steroid production. Histological studies on ovary and uterus also demonstrated dose dependent and time dependent structural changes, wherein cadmium and combined treated group showed maximum change which is correlated with the accumulation of lead and cadmium in the organs. Such a dose dependent or time dependent increase has not been observed in enzyme activities suggesting saturation at the active site of the enzymes by the metals after a dose of 0.05 mg/kg body wt. and 30 days treatment.

From dose and time dependent studies, 0.05 mg/kg body wt. and 15 days exposure were selected as the optimum dosage regime for all further experiments. Pregnancy is a main physiological stage of female reproductive cycle, where several physiological changes are known to occur. Therefore, it was of great interest to understand the effect of both lead and cadmium either alone or in combination during pregnancy. Reproductive performance in terms of body weight, pregnancy rate, litter size, placental and ovarian weights were not altered in all metal treated groups. But biomolecules like DNA, RNA, protein and glycogen was decreased in all metal treated groups, which is similar to the observations reported by other workers (Antonio et al.,

1999; Corpas et al., 1996). A significant inhibition of key steroidogenic enzymes, 3β HSDH and 17β HSDH activities were observed in both steroidogenic organs-ovary and placenta along with the decrease in steroid hormones. Decreased steroid production in the ovaries and placenta is in agreement with several other reports (Laskey and Piasek, 1994; Piasek et al., 2002). Total lipid and cholesterol content were decreased in metal treated groups, which is related to decreased expression of LDL-receptor (Joliobios et al., 1999), which could partly be responsible for decreased progesterone synthesis as observed in our study. Cadmium and combined treated group showed maximal effect compared to lead group. These effects are well correlated with the accumulation of both lead and cadmium in ovary and placenta. It is clearly indicated in literature that lead can easily pass through the placenta while cadmium is impermeable (Lucis et al., 1972; Arizono et al., 1981). Adaptive mechanism like metallothionein induction occurs on cadmium exposure and helps in sequestering metal ions (Goyer and Cherian, 1998), by displacement of zinc. Such an induction, with effective displacement was observed in placenta of cadmium and combined treated animals in the present study. Inspite of metallothionein induction, greater effect is manifested in cadmium and combined treated animals, indicating saturation of metal at the sulfhydryl group of metallothionein protein. As placental membrane allows a free passage to lead and cadmium gets accumulated. Combined treated animals demonstrated similar placental effects as in cadmium treated animals.

It is clearly indicated that lead and cadmium, both in isolation and in combination affects steroidogenesis in both non-pregnant and pregnant stages of reproductive cycle. Granulosa cells are the important ovarian cells that control follicogenesis and milieu of ovulation. Hence, granulosa cells was chosen as the cellular model to understand the

mechanism behind decreased steroid production. Peptide hormones, Follicle stimulating hormone (FSH) and Luteinizing Hormone (LH) binds to their specific receptors which are localized on cell membrane of the granulosa cells, to mediate the production of gonadal steroids by steroidogenesis. A significant decrease in binding of gonadotropins were observed in all metal treated groups wherein cadmium treated animals exhibited maximum decrease while lead treated animals showed minimal change. The pattern of inhibition of 17β HSDH activity was similar to changes observed in gonadotropin binding. Similar change in steroid production was reported earlier by several workers (Paksy et al., 1992; Piasek and Laskey, 1999).

From above, it is very clear that lead and cadmium both either in isolation or in combination inhibits steroid production in granulosa cells, through decreased binding of gonadotropins and by inhibiting steroidogenic enzymes In order to understand the mechanism of decreased binding leading to decreased steroid production, both "in *vivo*" and "*in vitro*" studies were done. It is clearly known that the membrane of steroid producing cells contains the receptors for gonadotropins and hence maintenance of membrane integrity is very important. Proposed mechanism by which the metals show their toxic effects is by binding to sulfhydryl groups and affecting the antioxidant enzymes, leading to an increase in free radicals. Our study also demonstrates that metals causes a change in membrane integrity along with increased lipid peroxides and altered antioxidant system in both granulosa cell and placenta. This alteration in membrane integrity has also reflected a change in activity of membrane bound enzymes namely Na^+ K^+ ATPase in all the metal treated groups. This indicates that "*in vivo*" decreased steroid production is due to multiple factors that includes change in membrane integrity

due to increase free radicals, which in turns causes an alteration in receptor structure leading to change in hormone binding, thereby resulting in decreased steroidogenesis. " *In vitro"* metal exposure did not alter the membrane integrity and antioxidant enzymes, but caused a decreased gonadotropin binding and steroidogenesis. However, the decrease observed was less than " *in vivo* " exposure. This suggests there exists a direct effect of metals on gonadotropin receptors and steroidogenic enzymes, which could be due to binding of metals at the sulfhydryl groups of amino acid residues. This was confirmed by pretreatment with zinc or sulfhydryl protectant (GSH, DTT) which could ameliorate the receptor binding and enzyme activity. This study indicates that lead and cadmium exerts its effects, by both primary and secondary effects on ovarian function. This study indicates that low level simultaneous exposure of lead and cadmium possess a threat to reproductive health of women.