List of figures and tables

Chapter 1

Figures	Title	Page #
1	The hypothalamic-pituitary-gonadal axis.	3
2	Structure of adult ovary	8
3	The major histological of preantral folliculogenesis	9
4	The early differentiation of the granulosa cells during preantral folliculogenesis involves the expression of FSH receptors.	10
5	Photomicrograph of an early tertiary follicle at the early antrum stage.	11
6	Schematic overview of the apoptotic process in follicular granulosa cells	14
7	Factors that regulate the stage-dependent survival of ovarian follicles.	15
8	FSH signal transduction pathway in granulosa cells of a dominant follicle	18
9	The regulatory mechanisms of androgen production by theca interstitial cells	20
10	Two Gonadotropin-Two Cell Concept	21
11	Functions of Steroidogenic Acute Regulatory Protein (StAR)	23
12	Steroidogenic Pathways	25
13	Estrogen synthesis and metabolism	30
14	Metal clusters of metallothionein	39
15	Role of oxidative stress in male and female fertility	42
Tables	Title	Page #
1	Main classes of EDCs present in food and in environment	32

Tables	litte	rage #
1	Main classes of EDCs present in food and in environment with major relevance for female reproductive system	32
2	Signs and symptoms associated with lead toxicity	37

Chapter 2

Tables			Title			A.A		Page#
1	Sequences	of	oligonucleotide	primers	used	for	PCR	57
	amplificatio	n						

Figures	Title	Page #
1	Effect of Gestational and Lactational exposure to lead and cadmium in isolation and combination on Dopamine levels in PND56 male rats (A) and PND56 female rats (B)	101
2	Effect of Gestational and Lactational exposure to lead and cadmium in isolation and combination on norepinephrine levels in PND56 male rats (A) and PND56 female rats (B)	101
3A	Effect of gestational and lactational co-exposure to lead and cadmium on the mRNA expression of (A) GnRH gene and (B) β -actin (internal control).	102
3B	Effect of gestational and lactational co-exposure to lead and cadmium on mRNA expression of LH &FSH genes and β -actin (internal control)	102

Tables	Title	Page #
1	Body weight, absolute and relative hypothalamus, pituitary weights of PND 56 F1 offspring after gestational and lactational exposure to lead and cadmium alone and in combination	97
2	Lead and Cadmium levels in hypothalamus of PND 56 F1 offspring after gestational and lactational exposure to lead and cadmium alone and in combination	98
3.	Lead and Cadmium levels in pituitary of PND 56 F1 offspring after gestational and lactational exposure to lead and cadmium alone and in combination	98
4	Effect of gestational and lactational exposure to lead and cadmium on steroid metabolizing enzyme activity in hypothalamus and pituitary of PND 56 rats	100
5	Effect of gestational and lactational co-exposure to lead and cadmium on the non-enzymatic and enzymatic antioxidants of hypothalamus in PND 56 rats	103
6	Effect of gestational and lactational exposure to lead and cadmium on the non-enzymatic and enzymatic antioxidants of pituitary in PND56 rats	104

Figures	Title	Page #
1	Effect of gestational and lactational co-exposure to lead and cadmium on ovarian 17β -hydroxysteroid dehydrogenase activity in PND 56 rats	115
2	Effect of gestational and lactational co-exposure to lead and cadmium on ovarian 3β -hydroxysteroid dehydrogenase activity in PND 56 rats	115
3	Effect of gestational and lactational co-exposure to lead and cadmium on the mRNA expression levels of ovarian (A) StAR gene (B) CYP11 and (C) β -actin (internal control)	116
4	Effect of gestational and lactational co-exposure to lead and cadmium on basal level Ovarian StAR protein expression by western-blot analysis. (A) Representative western immunoblot of ovarian StAR protein. (B) Composite graph showing the mean (±SEM) densitometric quantitation of the bands from three blots corresponding to the StAR protein	117
5	Effect of gestational and lactational co-exposure to lead and cadmium on serum estradiol levels in PND 56 rats	118
6	Effect of gestational and lactational co-exposure to lead and cadmium on serum progesterone levels in PND 56 rats	118
7	Histology of PND 56 ovary after gestational and lactational exposure to lead and cadmium alone and in combination	120
8	Effect of gestational and lactational exposure to lead and cadmium on reduced glutathione content in ovary of F1 generation PND 56 rats	121
9	Effect of gestational and lactational exposure to lead and cadmium on TBARS levels in ovary of F1 generation PND 56 rats	121

Tables	Title	Page #
1	Effect of Gestational & Lactational Exposure to lead and cadmium in isolation & combination on reproductive performance of dams	112
2	Effect of gestational and lactational exposure to lead and cadmium on body weight, ovary and uterus weights in F1 generation PND 56 rats	113
3	Lead and cadmium levels in the ovary of PND 56 F1 offspring after gestational and lactational co-exposure to lead and cadmium	113
4	Effects of gestational and lactational co-exposure to lead and	113

Γ		cadmium on the biochemical parameters of Ovary of PND 56	
		rats	
	5	Effects of gestational and lactational exposure to lead and	122
		cadmium on enzymatic antioxidants in ovary of PND 56 rats	

*

Figures	Title	Page #
1	Effect of gestational and lactational exposure to lead and cadmium on testicular 17β - hydroxysteroid dehydrogenase activity of PND 56 rats	132
2	Effect of gestational and lactational exposure to lead and cadmium on testicular 3β -hydroxysteroid dehydrogenase activity of PND 56 rats	132
3	Effect of gestational and lactational co-exposure to lead and cadmium on basal level testicular StAR protein expression by western-blot analysis. (A) Representative Western immunoblot of StAR protein in testis (B) Composite graph showing the mean (±SEM) densitometric quantitation of the bands from three blots corresponding to the StAR protein	133
4	Effect of gestational and lactational co-exposure to lead and cadmium on the expression of testicular (A) StAR gene and (B) CYP 11 gene in PND 56 testis	134
5	Effect of gestational and lactational exposure to lead and cadmium on serum testosterone levels of PND 56 rats	135
6	Histology of Testis	138

Tables	Title	Page #
1	Effect of gestational and lactational exposure to lead and cadmium on body weight, testis, and epididymis weights in PND 56 Male rats	130
2	Lead and Cadmium levels in testis of PND 56 F1 offspring after gestational and lactational exposure to lead and cadmium alone and in combination	130
3	Effect of gestational and lactational exposure to lead and cadmium on biochemical parameters in testis, cauda epididymis and seminal vesicle of PND 56 rats	131
4	Effect of gestational and lactational exposure to lead and cadmium on testicular and cauda epididymal sperm numbers, % sperm motility of PND 56 rats	136
5	Effect of gestational and lactational exposure to lead and	136

	cadmium on testicular and cauda epididymal non-enzymatic antioxidant and lipid peroxidation	
6	Effect of gestational and lactational exposure to lead and cadmium on testicular and cauda-epididymal enzymatic antioxidants	137

Figures	Title	Page #
1	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on NADPH- cytochrome c reductase activity in PND 56 female rats (A) and male rats (B)	149
2	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on NADH- cytochrome c reductase activity in PND 56 female rats (A) and male rats (B)	149
3	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on γ -glutamyl transferase activity in PND 56 female rats (A) and male rats (B)	150
4	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on UDP-glucoronyl transferase activity in PND 56 female rats (A) and male rats (B)	150
5	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on Glutathione-S-transferase activity in PND 56 female rats (A) and male rats (B)	151
6	Effect of gestational and lactational exposure to lead and cadmium alone and in combination on 17β -hydroxysteroid oxidoreductase activity in PND 56 female rats (A) and male rats (B).	151
7	Histology of PND 56 liver of female rats	152

Tables	Title	Page #		
1	Body weights, absolute liver weights of PND 56 F1 offspring after gestational and lactational exposure to lead and cadmium alone and in combination			
2	Lead and Cadmium contents in liver of PND 56 F1 offspring after gestational and lactational co-exposure to lead and cadmium			

3	Effect of gestational and lactational exposure to lead and cadmium on markers of general toxicity in PND 56 rats	153
4	Effect of gestational and lactational exposure to lead and cadmium on the non-enzymatic antioxidant and TBARS level of liver in PND 56 rats	153
5	Effect of gestational and lactational exposure to lead and cadmium on enzymatic antioxidants of liver in PND 56 rats	154
6	Effect of gestational and lactational exposure to lead and cadmium on the biochemical parameters (Cholesterol, Glycogen, DNA and RNA) of liver in PND 56 rats	155

Chapter 4 Section 4.2

Figures	Title	Page #
1	Effect of pubertal co-exposure to lead and cadmium on hypothalamic 3α-hydroxy steroid dehydrogenase activity in PND56 female rats	172
2	Effect of pubertal co-exposure to lead and cadmium on pituitary 3α -hydroxy steroid dehydrogenase activity	172
3	Effect of pubertal co-exposure to lead and cadmium on hypothalamic dopamine levels in PND56 female rats	173
4	Effect of pubertal co-exposure to lead and cadmium on hypothalamic norepinephrine levels in PND56 female rats	173
5A	Effect of pubertal co-exposure to lead and cadmium on mRNA expression level of hypothalamic GnRH gene (a) GnRH (b) β -actin (internal control) (c) relative mRNA expression levels (arbitrary units)	174
5B	Effect of pubertal co-exposure to lead and cadmium on mRNA expression level of pituitary LH and FSH genes (a) LH (b) FSH (c) β -actin (internal control) (d) relative mRNA expression levels (arbitrary units)	175
6	Effect of pubertal co-exposure to lead and cadmium on hypothalamic reduced glutathione levels in PND 56 female rats	177
7	Effect of pubertal co-exposure to lead and cadmium on hypothalamic TBARS levels in PND56 female rats	177
8	Effect of pubertal co-exposure to lead and cadmium on pituitary reduced glutathione levels in PND56 female rats	178
9	Effect of pubertal co-exposure to lead and cadmium on pituitary TBARS levels in PND56 female rats	179

Tables	Title	Page #		
1	Effect of pubertal co-exposure to lead and cadmium on Body weight, Absolute and Relative weights of hypothalamus, pituitary			
2	Lead and Cadmium levels in hypothalamus and pituitary of PND 56 female rats	170		
3	Effect of pubertal co-exposure to lead and cadmium on hypothalamic enzymatic antioxidants	178		
4	Effect of pubertal co-exposure to lead and cadmium on pituitary enzymatic antioxidants in PND56 female rats	179		

•

Chapter 4 Section 4.3

Figures	Title	Page #
1	1 Effect of pubertal co-exposure to lead and cadmium on (A) ovarian 17β-hydroxy steroid dehydrogenase activity and (B) 3β-hydroxy steroid dehydrogenase activity.	
2	Effect of pubertal co-exposure to lead and cadmium on the mRNA expression levels of ovarian (A) StAR, (B) CYP 11, (C) CYP19 (D) 3β -HSD and β - Actin (internal control)	191
3	Effect of pubertal co-exposure to lead and cadmium on basal level ovarian StAR protein expression by western-blot analysis	192
4	Effect of pubertal co-exposure to lead and cadmium on serum estradiol levels (A) and serum progesterone levels (B)	192
5	Histology of PND 56 Ovary	193
Tables	Title	Page #
1	Effect of pubertal co-exposure to lead and cadmium on body weights and organ weights of PND 56 rats	187
2	Lead and Cadmium levels in PND 56 ovary after pubertal co- exposure to metals	187
3	Effect of pubertal co-exposure to lead and cadmium in isolation and in combination on ovarian total granulose cell count of PND 56 rats	188
4	Effect of pubertal co-exposure to lead and cadmium in isolation & combination on biochemical parameters of PND 56 ovary	188

5	Effect of Pubertal co-exposure to lead and cadmium on reduced glutathione (GSH) and TBARS levels in PND 56 ovary	194
6	Effect of pubertal co-exposure to lead and cadmium on enzymatic antioxidants of PND 56 ovary	194

.

Chapter 4 Section 4.4

Figures	Title	Page #			
1	1 Effect of Pubertal exposure to lead and cadmium alone and in combination on NADPH- cytochrome c reductase activity in PND 56 female rats				
2	Effect of pubertal exposure to lead and cadmium alone and in combination on NADH- cytochrome c reductase activity in PND 56 female rats.				
3	3 Effect of pubertal exposure to lead and cadmium alone and in combination on Gamma-glutamyl transpeptidase (γ-GT) activity in PND 56 female rats				
4	Effect of Pubertal exposure to lead and cadmium alone and in combination on UDP-Glucoronyl transferase activity (UDPGT) in PND 56 female rats	206			
5	Effect of pubertal exposure to lead and cadmium alone and in combination on Glutathione-S-transferase (GST) activity in PND 56 female rats	207			
6	Effect of Pubertal exposure to lead and cadmium alone and in combination on 17β -hydroxy steroid oxidoreductase (17β -HSOR) activity in PND 56 female rats	207			
7	Histopathology of PND 56 Liver of female rats	208			
8	Figure 8: Effect of pubertal exposure to lead and cadmium alone and in combination on reduced glutathione levels (GSH) in PND 56 female rats				
9	9 Effect of pubertal exposure to lead and cadmium alone and in combination on lipid peroxidation levels in PND 56 female rats				
Tables	Title	Page #			
1 Effect of pubertal exposure to lead and cadmium on the body weight, absolute liver weight and relative weight of PND 56 female rats					

•

2	Metal accumulation in liver of PND56 rats after pubertal exposure	204
3	Effect of pubertal exposure to lead and cadmium on the biochemical parameters (Cholesterol, Glycogen, DNA, and RNA) of liver in PND56 female rats	209
4	Effect of pubertal exposure to lead and cadmium on enzymatic antioxidants of liver in PND56 female rats	211

Chapter 5

Figures	Title	Page #
1	Effect of <i>"in vivo"</i> exposure to lead and cadmium in isolation and combination on 17β -hydroxysteroid dehydrogenase activity of granulose cells in gonadotropin primed immature rats	226
2	Effect of in vivo exposure (PND 21 to PND 25) to lead and cadmium in isolation and combination on (A) serum estradiol and (B) progesterone levels in gonadotropin primed immature female rats	226
3	Effect of " <i>in vivo</i> " exposure (PND21 to PND25) to lead and cadmium in isolation and combination on reduced glutathione (GSH) levels of luteinized granulosa cells from gonadotropin primed immature rats	227
4	Effect of <i>"in vivo"</i> exposure (PND 21 to PND 25) to lead and cadmium in isolation and combination on TBARS levels of luteinized granulosa cells from gonadotropin primed immature rats	227
5	Effect of <i>"in vivo"</i> exposure (PND 21 TO PND 25) to lead and cadmium in isolation and combination on Catalase activity of luteinized granulose cells obtained from gonadotropin primed immature rats	228
6	Effect of " <i>in vivo</i> " exposure (PND 21 to PND 25) to lead and cadmium in isolation and combination on SOD activity of luteinized granulose cells obtained from gonadotropin primed immature rats	228
7	Effect of <i>"in vitro"</i> exposure to lead and cadmium on % viability of luteinized granulosa cells after 6 h exposure at $(50\mu M \& 200\mu M)$ concentration of metals alone and in combination.	231

8	Effect of <i>"in vitro"</i> exposure to lead and cadmium on % viability of luteinized granulosa cells after 12 h exposure at $(50\mu M \& 200\mu M)$ concentration of metals alone and in combination.	231
9	Effect of " <i>in vitro</i> " exposure to lead and cadmium (6 h exposure at concentrations of 50µM & 200µM) on estradiol levels in conditioned media of luteinized granulosa cells isolated from gonadotropin primed immature rats.	232
10	Effect of " <i>in vitro</i> " exposure to lead and cadmium (12 h exposure at concentrations of 50μ M & 200μ M) in isolation and combination on estradiol levels in conditioned media of luteinized granulosa cells isolated from gonadotropin primed immature rats.	232
11	Effect of " <i>in vitro</i> " exposure to lead and cadmium (6 h exposure at concentrations of 50μ M & 200μ M) on progesterone levels in conditioned media of luteinized granulose cells isolated from gonadotropin primed immature rats.	233
12	Effect of " <i>in vitro</i> " exposure to lead and cadmium (12 h exposure at concentrations of 50μ M & 200μ M) on progesterone levels in conditioned media of luteinized granulose cells isolated from gonadotropin primed immature rats.	233
13	Effect of " <i>in vitro</i> " exposure to lead and cadmium (12 h exposure at concentration of 200μ M) on 17β -hydroxysteroid dehydrogenase activity in luteinized granulose cells isolated from gonadotropin primed immature rats.	234
14	Effects of " <i>in vitro</i> " exposure to lead and cadmium (12 h exposure at concentrations of 50μ M and 200μ M) in isolation and in combination on caspase-3 activity in the luteinized granulose cells from gonadotropin primed immature rats.	235
15	Effects of " <i>in vitro</i> " exposure to lead and cadmium for 12 h at concentrations of (50μ M and 200μ M) in isolation and in combination on % LDH release measured in conditioned media of luteinized granulose cells.	235
16	Luteinized granulose ROS levels, as measured by DCF	237

,

•	· ·		

fluorescence intensity, increase upon incubation with lead and cadmium for 12 h at the concentration of 200µM. 17 Comet staining of DNA from luteinized granulose cells after being exposed to lead and cadmium for 12 h at concentration of 200µM in isolation and in combination. 239 18 Effect of "in vitro" exposure to lead and cadmium on mRNA expression of StAR, 17β-HSD, Cyp19 and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to 200 µM concentration of each metal. 241 19 Effect of "in vitro" exposure to lead and cadmium on mRNA expression of progesterone receptor, FSH receptor, IGF-1, and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to metals alone and in combination.(ND=not detected) 243 20 Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination 243 20 Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination 243 21 Effect of "in vivo" exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats. 243 3 Effect of "in vitro" exposure to lead and cadmium in isolation and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats. (200µM, 12 h). 236			
being exposed to lead and cadmium for 12 h at concentration of 200μM in isolation and in combination.24118Effect of "in vitro" exposure to lead and cadmium on mRNA expression of StAR, 17β-HSD, Cyp19 and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to 200 μM concentration of each metal.24119Effect of "in vitro" exposure to lead and cadmium on mRNA expression of progesterone receptor, FSH receptor, IGF-1, and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to metals alone and in combination.(ND=not detected)24220Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination24320Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination24321Effect of "in vitro" exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats.2432Effect of "in vivo" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2243Effect of "in vivo" exposure to lead and cadmium in isolation and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats.236			
expression of StAR, 17β-HSD, Cyp19 and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to 200 μM concentration of each metal.24219Effect of "in vitro" exposure to lead and cadmium on mRNA expression of progesterone receptor, FSH receptor, IGF-1, and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to metals alone and in combination.(ND=not detected)24320Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination243TablesTitlePage #1Effect of "in vivo" exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats.2242Effect of "in vivo" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.224	17	being exposed to lead and cadmium for 12 h at concentration	239
expression of progesterone receptor, FSH receptor, IGF-1, and β-actin (internal control) in luteinized granulosa cells after 12 h exposure to metals alone and in combination.(ND=not detected)20Effect of "in vitro" exposure to lead and cadmium on mRNA expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combination243TablesTitlePage #1Effect of "in vivo" exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats.2242Effect of "in vivo" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2243Effect of "in vitro" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.236	18	expression of StAR, 17 β -HSD, Cyp19 and β -actin (internal control) in luteinized granulosa cells after 12 h exposure to	241
expression of Bcl and Bax genes in luteinized granulosa cells after 12 h exposure to metals alone and in combinationPage #TablesTitlePage #1Effect of <i>"in vivo"</i> exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats.2242Effect of <i>"in vivo"</i> exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2243Effect of <i>"in vitro"</i> exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.236	19	expression of progesterone receptor, FSH receptor, IGF-1, and β -actin (internal control) in luteinized granulosa cells after 12 h exposure to metals alone and in	242
1Effect of "in vivo" exposure to lead and cadmium in isolation and combination on ovulation induced by gonadotropins in immature rats.2242Effect of "in vivo" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2243Effect of "in vivo" exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2363Effect of "in vitro" exposure to lead and cadmium in isolation 	20	expression of Bcl and Bax genes in luteinized granulosa cells	243
and combination on ovulation induced by gonadotropins in immature rats.22Effect of <i>"in vivo"</i> exposure to lead and cadmium in isolation and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2243Effect of <i>"in vitro"</i> exposure to lead and cadmium in isolation and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats.236	Tables	Title	Page #
and combination on body and reproductive organ weights, and ovulation in gonadotropin primed immature rats.2363Effect of "in vitro" exposure to lead and cadmium in isolation and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats.236	1	and combination on ovulation induced by gonadotropins in	224
and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats.	2	and combination on body and reproductive organ weights,	224
	3	and combination on antioxidant system in luteinized granulose cells from gonadotropin primed immature rats.	236