EFFECT OF HYPO- AND HYPERTHYROIDISM ON OVARIAN STRUCTURE AND FUNCTION IN RATS

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**DEGREE OF MASTER OF PHILOSOPHY
IN BIOLOGY**

REPRODUCTIVE PHYSIOLOGY

by

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CERTIFICATE

This thesis by Zertashia Akram is accepted in its present form by the Department of Biological Sciences, as satisfying the thesis requirements for the Degree of Master of Philosophy in Biology (Reproductive Physiology).

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ABSTRACT

The aim of present study was to examine the effects of hypo- and hyperthyroidism induced during pre- and postnatal period of life on ovarian function and structure in female rats at 120 days of age. Three chemicals were used in this study. In prenatal group, treatment was given from conception to parturition. While in postnatal group, treatment was given from parturition to 25 days postpartum. Hypothyroidism was induced by administration of 0.1% PTU in drinking water and by oral administration of 0.06mg malathion to mothers both for the pre- and postnatal treatment. Hyperthyroidism was induced by subcutaneous injection of 0.25µg thyroxine given to mothers in prenatal group, while in postnatal group 0.3µg/g BW (body weight) thyroxine was administered to the pups. Weekly body weight of the female pups were measured. In each group 10 female rats were sacrificed at 120 days of age.

Postnatal PTU treated pups show delay in eye opening, teething, fur development and weaning (35-37 days) compared to control (28-30 days). Body weight of female rats in postnatal PTU, pre-and postnatal thyroxine treatment group was significantly decreased ($P<0.001$) while, prenatal PTU treatment group showed significant increase ($P<0.0001$) compared to control. There was significant ($P<0.05$) reduction in paired ovarian weight of postnatal PTU and thyroxine treatment group compared to control. Malathion treatment caused no effect on body and ovarian weight. Diameter of the ovaries was not affected by any treatment. Regarding the morphometery, only prenatal PTU treatment showed a significant ($P<0.001$) increase in diameter of graafian follicles. No significant

difference was observed in morphometery of granulosa layer, primary and developing follicles of control and all treated groups. Number of primary, developing and graafian follicles of all the treated group were similar to that of control. The corpora lutea of postnatal PTU and thyroxine treated group contained a population of large numbers of luteal cells compared to control. All other treated groups have no profound effect on ovarian morphology, histology and morphometery. No difference was found in serum estradiol concentration of control, pre- and postnatal PTU, malathion and thyroxine treated groups.

In summary it is concluded from the present study that successful treatment of 0.1% PTU to immature female rats from 0-25 day postpartum effect the growth, physical development caused a decrease in body weight and ovarian weight at 120 day of age. Treatment of immature rats with $0.3\mu g$ thyroxine/g BW from 0-25 day postpartum also decrease the body and ovarian weight. This indicate the importance of thyroid hormone during the early postnatal period of life regarding the growth, physical development and ovarian weight. While pre- and postnatal malathion and prenatal PTU and thyroxine have no profound effect on ovarian morphology, histology and morphometery. All the treatment during the pre- and postnatal period of life do not alter the concentration of estradiol at 120 day of age.

INTRODUCTION

It is well established fact that thyroid hormones play an important role in embryonic or foetal development of vertebrates (Sullivan et al., 1987). Normal sexual and reproductive function depends upon thyroid activity and its secretion (Guyton, 1996; Turner and Bagnara, 1976). Thyroid activity is studied in different species with respect to development and reproduction. For example, it plays an important role in regulating the onset of puberty and reproductive function in birds (Kirby et al., 1996). Deficiency or excess of thyroid hormones (hypothyroidism and hyperthyroidism) induces abnormalities in reproduction (Mattheij et al., 1995; Kalland et al., 1978). Hypothyroidism appears to delay sexual development in young male and toxic levels of thyroxine appear to impair reproductive function (Turner and Bagnara, 1976). Hypothyroidism in rats results in fewer pregnancies and reduction in litter size (Varma et al., 1978). Many of the young die because of insufficient lactation by mothers (Turner and Bagnara, 1976).

It was observed by Maia et al (1990) that reproductive system of the immature rat is held to be more influenced by thyroid dysfunction than that of the adult. Hypothyroidism in rats has been shown to adversely affect foetal development (Kalland et al., 1978). Presence of atrophied and under weight ovaries in hypothyroid rats were observed by Ortega et al (1990); Turner and Bagnara (1976). While, Fitko and Szlezyngier (1994) studied the effect of hyperthyroidism and found that ovaries of hyperthyroid rats were diminished in size.

Interference between function of the thyroid gland and the ovary was studied in humans by Wurfel (1992). He noticed that thyroid disorders may influences

ovarian cycle, secretion of GnRH i.e, FSH and LH, steroid metabolism (androgen and estrogen) and prolactin (PRL) metabolism. Mattheij et al (1995) observed the effect of thyroid hormones on reproductive organs of female rats. They induced hypothyroidism in female rats by thyroidectomy, and found that ovaries of hypothyroid rats contain more large atreitic follicles whose steroid metabolism was also disturbed. In addition, in these hypothyroid female rats the disturbed steroid metabolism both in the growing follicles and in the corpora lutea causing the prolongation of luteal phase. They concluded that deficiency of thyroid hormone decreases the ovulation rate, because hypothyroidism reduced the number of follicles which are able to ovulate.

Thyroid hormone imbalance effects other developmental process in different species. For example, adult hypothyroid human and mice can loose their sense of smell (Beard and Mackay-Sim, 1987; Mackay-Sim and Beard, 1987). Hypothyroidism in rats results in reduced body weight (Madeira et al., 1991; Madeira et al., 1992; Madeira and Paula-Barbosa, 1993). It delays but does not abolish weaning process (Blake et al., 1985) causes CNS abnormalities (Akaike and Kato, 1997) and also produces general inattention to the environment (Rial et al., 1987).

Hypothyroidism can be induced experimentally by thyroidectomy or an antithyroid drug. While the severity of hypothyroidism induced by thyroidectomy is hard to regulate, the degree of hypothyroidism induced by antithyroid drug can be easily controlled by changing the dose of drug (Akaike and Kato, 1997).

PROPYL THIOURACIL (PTU):

6-n-propyl-2-thiouracil (PTU) (Hardy et al; 1996) is a derivative of thiocarbamide (Turner and Bagnara, 1976). It is an antithyroid (Yang and Gorden, 1997) reversible goitrogen drug (Cooke et al., 1992; Kirby et al., 1992; Mendis-Handagama and Sharma, 1994; Hardy et al., 1996). PTU is a potent inducer of hypothyroidism in the rats (Blake et al.,1985). It is effective in producing hypothyroidism early in the life (Guedes and Pereira-Da-Silva, 1993). PTU is transferred from mothers to their pups through placenta (Marchant et al., 1977; Mortimer et al., 1997) and milk (Kawada et al., 1988). Administration of PTU decreases serum thyroxine and triiodothyronine levels during the postnatal period of life which was indicative of severe hypothyroidism (Van Haaster et al.,1992; Cooke et al.,1993; Yang and Gorden 1997).

PTU is in white powdered form and has bitter taste. Effects of PTU treatment has been studied in birds, humans and rats. PTU is effective and safe in the treatment of hyperthyroidism in pregnancy in human (Mortimer et al., 1997; Deborah et al., 1994). In domestic fowl, PTU treatment may result in permanent increase in testes size and DSP (Kirby et al., 1996).

Cooke et al (1993) studied in rats, that water uptake of the mothers offered water containing 0.1% PTU from 0-25 day postpartum is markedly reduced immediately after parturition and remains reduced until the end of suckling period.

It has been observed by Maia et al (1990) that reproductive system of immature rat is held to be more influenced by thyroid dysfunction than that of adult. Dijkstra et al (1996) studied the effect of prepubertal hypothyroidism on ovarian development in rats. Hypothyroidism was induced by giving 0.1% PTU in drinking water of mothers and pups from birth to day 40 postpartum. They found that hypothyroidism of immature female rats resulted in decreased body and ovarian weight. At day 40, they observed that ovaries of PTU treated rats contained more secondary and less antral follicles, smaller non-atretic antral follicles and more atretic follicles as compared to control. While corpora lutea were absent at day 40. This disturbed folliculogenesis further affecting the follicular development by influencing the granulosa cell differentiation but not their proliferation. They concluded that this disturbed folliculogenesis is due to inadequate thyroid hormone supply which lead to anovulation.

Effect of hypothyroidism was also observed on reproductive system of female mice. Chan and Ng (1995) induced hypothyroidism by giving subcutaneous injection of 50μg/g BW PTU from postnatal day 1 onward. No difference was observed on ovarian histology between control and PTU treated mice at day 14, 21 and 28. While postnatal hypothyroidism affect the number of ovarian follicles at different maturation stages. There was reduction in the number of graafian follicles and multilaminar follicles in PTU treated mice studied at postnatal day 14, 21 and 28 as compared to control. At postnatal day 28 Chan and Ng (1995) also

observed dead follicular cells around the oocyte, although the oocyte themselves appeared to be normal.

Different scientists studied the effect of PTU during postnatal period of life and reported , that the postnatal PTU treated pups have retarded growth and physical development, delay in eye opening and teething, slow in responding to general environment and also have depressed body weight as compared to control (Meisami, 1984; Tamasy et al., 1984; Kawada et al., 1988; Akaike et al., 1990; Madeira et al., 1991; Cooke et al., 1992; Madeira et al., 1992; Madeira and Paula-Barbosa, 1993; Akaike and Kato, 1997).

MALATHION:

Malathion (0,0- dimethyl- (1,2- dicarbethoxyethyl) dithiophosphate (Pluth et al., 1996) is an insecticide (Akhtar et al., 1996; Lechner et al., 1984; Husain et al., 1989) belonging to the group of organophosphorus (Balasubramanian et al., 1987; Seal, 1988; Akhtar et al., 1996). Organophosphate compounds are widely used in the control of pests (Samaan et al., 1989; Ozmen and Akay, 1993). Likewise, malathion is used for both domestic and commercial agriculture purposes.(Pluth et al., 1996). Its residues have been detected in various food stuffs (Balasubramanian et al., 1986). It has been observed by Akhtar et al (1996) that administration of 0.06mg. malathion for 21 days to adult rats caused a decrease in serum thyroxine and triiodothyronine levels. Balasubramanian et al (1986) have reported that malathion, depending upon the dosage, route and

administration resulted in altered thyroid function in rats. Therefore, malathion is used to induce hypothyroidism in rats. Akhtar et al (1996) observed no effect of 0.06mg. mala- thion on body weight of adult rats. Samaan et al (1989) compared the toxicity of malathion, methomyl and pyrethroid fenvalerate in rats. Their study demonstrates that tested insecticides induced marked and dose dependent growth retardation, among which, malathion was least toxic.

There has also been some evidence that malathion have a teratogenic effect (the ability to cause birth defect). The offspring of rats fed with 240mg/Kg/day mala- thion resulted in growth retardation and elevated mortality, although no negative effect has been observed in the parents. (Pluth et al., 1996).

Effect of malathion has also been studied in mouse by Mufti and Asmatullah (1997). They used different doses of malathion (55.84, 111.67 and 233.34 µg/g BW) which were orally administered to mothers on different gestation days. The pups were removed at 15 day of gestation. They found that malathion caused a dose dependent foetal mortality. Highest foetal mortality, and in some cases, total resorption, and also decreased body weight of foetus was observed by the administration of highest dosage of malathion. While, lower doses caused overall reduction in body size, but other organ differentiation was normal in comparison to control. Polydactyly was also noticed with different doses of malathion. Therefore, they concluded that high doses of malathion are embryotoxic and teratogenic in mice. Toxicity of malathion was observed by Ozmen and Akay (1993). They used two dosage levels of malathion (10 and 100mg/Kg) given to rats daily for 15

weeks. No histopathological changes have been observed in the ovaries of the rats. No difference was observed in serum estradiol levels between control and both treatment groups.

THYROXINE:

Scanty information are available regarding the effect of thyroxine on female reproductive function and structure. The thyroid has greater capacity than any other endocrine gland for storing its secretions (Gentile et al., 1995). Major role of thyroid gland is the production of thyroxine and triiodothyronine. Thyroxine is highest in concentration and is the only one that arise solely by direct secretion from the thyroid gland (Larsen and Ingbar, 1992). The normal plasma concentration of thyroxine increases rapidly after birth and is maximal during the third week of life (Clos et al., 1974). Thyroxine along with thyroid stimulating hormone (TSH) are used as biochemical indicator of thyroid function, which aids the diagnosis and monitoring of either hyperthyroidism and hypothyroidism (Guyton, 1996).

Thyroxine is primarily a growth and differentiation promoting hormone which also control the rate of metabolic process in the body and influencing the physical development (Turner and Bagnara, 1976). Thyroxine is essential for the normal development of foetus and differentiation of the CNS (Johnson and Everitt, 1995). Importance of thyroid hormones regarding the growth and development is also reported by Coulombe et al (1980). Thyroxine repair the pituitary defects in the

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thyroidless rats. In the hypophysectomized animal, thyroxine increases the rate of oxygen consumption, accelerates the heart rate, but it does not restore the normal body growth or reproductive development (Turner and Bagnara, 1976; Gill, 1991).

It is observed by Besa and Pascual-Leone (1984) that administration of high doses of thyroxine to rats during their first week of life results in decreased body and gonadal weight. Decreased ovarian size in hyperthyroid rats is also noticed by Fitko and Szlezyngier (1994). Van Haaster et al (1993) induced hyperthyroidism in rats by means of triiodothyronine and found decrease in their body and gonadal weights of these rats at 100 day of age. Toxic levels of thyroxine impair reproductive function (Turner and Bagnara, 1976). It has been suggested by Mestman (1997) that hyperthyroid women should be renderd euthyroid before conception. Because the incidence of maternal and neonatal morbidity is significantly higher in those patients whose hyperthyroidism is not medically controlled.

Thyroxine is also used for the treatment of hypothyroidism (Gill, 1991). Treatment with 0.20 or 1.50µg thyroxine/100g BW/day to rats, partially reversed the changes induced by Juvenile-onset hypothyroidism (Marcos et al., 1994). Clos et al (1974) noticed that in young male rats, the concentration of thyroxine in the plasma progressively increases from birth to the third postnatal week. Thyroxine turnover is significantly higher during this period. They found that body weight of hypothyroid rats was completely restored by administration of thyroxine (0.20 and 0.25µg/day) during the first three postnatal weeks. It remains controversial that

whether there is significant transfer of thyroid hormone in a normal pregnancy in any species or not (Porterfield and Hendrich, 1981).

The objective of the present study is to examine the effect of hypothyroidism induced by PTU, malathion and hyperthyroidism by thyroxine during the prenatal and postnatal period of life on ovarian morphology, histology, morphometery and serum estradiol concentration in rats at 120 day of age.

MATERIALS AND METHODS

Male and female Sprague-Dawely rats were obtained from National Institute of Health (NIH) Islamabad, and maintained at the Animal House of Biology Department, Q.A.U, Islamabad. Rats were kept under standard laboratory conditions, fed standard diet and received tap water ad libitum. After mating, female rats were caged separately. Just before the initiation of experiment, female rats were weighed and their body weight ranged from 210-240gm.

Female rats were randomly divided into three groups, Control, Prenatal and Postnatal group. Three different chemicals were used in this study to induce hypo- and hyperthyroidism. Therefore, six groups were designed on the basis of chemical and duration of treatment. Total seven groups were used in present study, one was control and six were treated groups, with five animal (n=5) in each group.

Three chemicals which were used in this study are given below:

a-PROPYL THIOURACIL(PTU):

6-n-propyl-2-thiouracil (Sigma-chemical Co. St. Louis USA) was used to induce hypothyroidism in female rats. 0.1% PTU /day (0.1gm. PTU dissolved in 95ml distilled water) in drinking water was given to mothers both in pre- and postnatal treatment group. 5ml. Rooh-Afza (Hamdard laboratories (WAQF), Lahore, Pakistan) was added to minimize the bitterness of PTU.

b-MALATHION:

Malathion (Fufanon 57% EC (cheminova Agro A/S; Lemvig Denmark) contain 57mg of Malathion-0,0-Dimethyl-s-[1,2-(ethoxycarbonyl) ethyl] phosphorodithio-ate per ml.) was used to induce hypothyroidism in female rats. 0.06mg. malahtion/ml of saline was orally administered by gastric tubulin to mothers of both pre- and postnatal treatment group.

c-THYROXINE:

Thyroxine Tablets (Glaxo Welcome Pakistan Ltd., Karachi, Pakistan) containing $50\mu g$ of thyroxine sodium B.P were used. One tablet was dissolved in 1ml. of distilled water. Subcutaneous injection of 0.25 μg thyroxine/day was given to pregnant rats in prenatal treatment group, while $0.3\mu g$ thyroxine/g body weight/day was given to pups (postnatal treatment group) to induce hypothyroidism.

One control and two major experimental groups were designed:

1. CONTROL:

No treatment was given to the mothers and their pups. Simple tap water and standard diet was given.

2. PRENATAL EXPERIMENTAL GROUP:

In this experiment treatment was given to dams from conception to the day of parturition. This experiment was further sub-divided into three groups on the basis

of chemicals. But duration of treatment was same for all the three chemicals. These three sub-groups were given below:

a-PTU prenatal treatment group.

b-Malathion prenatal treatment group.

c-Thyroxine prenatal treatment group.

3. POSTNATAL EXPERIMENTAL GROUP:

In this experiment treatment was given from parturition to 25 day postpartum. This experiment also contained three groups on the basis of chemicals. Treatment period was same for three chemicals. But PTU and malathion were given to mothers while, thyroxine was given to pups. Total three groups designed which were given below:

a PTU postnatal treatment group.

b-Malathion postnatal treatment group.

c-Thyroxine postnatal treatment group.

The pups from all the seven groups were weaned (25-30 days), weighed, sexed and housed 4-5 per cage. Only female pups were used in this study. Weekly feed consumption and water uptake of the female pups from all the seven groups were recorded from weaning to the end of experiment (120 days), by measuring the water remaining in drinking bottles and the remaining feed in the food box. Body

weight of pups from seven groups were recorded at weekly intervals through out the experiment (0-120 days).

COLLECTION OF BLOOD SAMPLE:

Blood was drawn from the aorta of the anaesthesized rats at 120 day of age by means of 5ml. sterilized syringe. All the blood sample were collected in glass centrifuged tubes. After chilling, samples were centrifuged at the rate of 2000 rpm. for 20 minutes. Serum was carefully collected with the help of pasture pipette and frozen for the estimation of estradiol by RIA.

SAMPLING:

At the age of 120 days, female rats from all the groups were randomly selected, weighed and sacrificed. Number of animals for each group was same (n=10). Ovaries were dissected out from each animal. Tissues debris were removed to make the organ clear. After which tissues were fixed for histological examination.

OVARIAN SIZE AND WEIGHT:

After removing the tissues debris, ovarian size was taken by Vernier Calliper. Paired ovarian weight (right + left ovary) was measured by using the Sarotoreious Digital Balance.

HISTOLOGICAL PROCESSING:

The ovaries from all the groups were fixed in sera fixative. Constitution of sera fixative is given below:

a- Absolute alcohol   =60ml.

b- Formaldehyde   =30ml.

c- Glacial acetic acid   =10ml.

After fixation tissues were processed for routine embedding in paraffin wax. The tissues were than sectioned (5μm, thick) and stained with hematoxylene and eosin (Mc Manus and Mowry, 1960; Drury and Wallington, 1980). Ocular micrometer was used at different magnification for Morphometeric analysis.

ESTIMATION OF ESTRADIOL:

Concentration of estradiol in serum was measured by using a specific radio-immunoassay (RIA) procedure. The RIA reagents were supplied by the World Health Organization's Special Program of Research in Human reproduction.300μl of serum extracted with 4ml of diethyl ether. Dried extracts were reconstituted with 1.0ml. of buffer steroid. 500μl samples and estradiol standard were incubated with 100μl estradiol antiserum and 100μl estradiol tracer (Tritiated 1-2-6-7 H³ estradiol) for 18h at 4°C. Next day 200μl of activated charcoal was added to each tube and them incubated for 30 minutes at 4°C. After incubation, tubes were centrifuged at 3000rpm for 15 minutes and supernatant was decanted in scintillation vials. In

each scintillation vial 5ml Scintillation cocktail was added. Radioactivity was counted in a Beckman (LS5801) liquid scintillation counter. Results of RIA were calculated by Logit-log transformation of data.

STATISTICAL ANALYSIS:

Data of all the seven groups were expressed as Mean ± SEM. To compare the control, pre- and postnatal treatment group one way-ANOVA, two way-ANOVA and Tukey's test was performed.

RESULTS

GENERAL OBSERVATIONS:

Litter size of PTU, malathion and thyroxine treated mothers, receiving treatment from conception to parturition or from parturition to 25 day postpartum are shown in Table 1A,1B; Figure 1A,1B. Mothers receiving PTU from conception to parturition have lower pregnancies (60%) and small litter size (15%) compared to control. These mothers have decreased feed consumption, water uptake and body weight during the treatment period compared to control.

In case of mothers receiving PTU from parturition to 25 days postpartum, it is observed that as the treatment starts they show reduction in feed and water consumption and start loosing their weights compared to control.

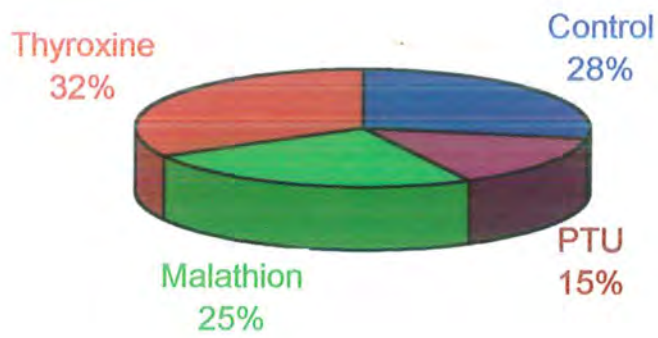Malathion and thyroxine treated mothers are normal in their pregnancies and litter size.

Postnatal PTU treated pups have normal body weight at the time of birth. It is observed that as the treatment is administered to mothers (parturition to 25 days postpartum) postnatal PTU treated pups start loosing their weight. Low survival rate (30%) of postnatal PTU treated pups is also observed. They also show retarded physical development, delayed eye opening, teething, fur development ,and show inattention to general environment. Postnatal PTU treated pups weaned between (35-37 days) later than control, malathion and thyroxine treated pups (28-30 days).

TABLE 1A: MEAN LITTER SIZE OF CONTROL AND TREARED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM CONCEPTION TO PARTURITION.

Groups	Litter Size		
n=5	Female Mean ± SEM	Male Mean ± SEM	Total Mean ± SEM
Control	6.00 ± 1.15	4.33 ± 1.76	10.33 ± 0.67
PTU	2.40 ± 0.68	3.00 ± 0.45	5.40 ± 0.68
Malathion	4.80 ± 1.02	4.40 ± 1.33	9.20 ± 1.53
Thyroxine	6.75 ± 0.63	5.50 ± 0.96	12.25 ± 0.63

TABLE 1B: MEAN LITTER SIZE OF CONTROL AND TREARED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM PARTURITION TO 25 DAY POSTPARTUM.

Groups	Litter Size		
n=5	Female Mean ± SEM	Male Mean ± SEM	Total Mean ± SEM
Control	6.00 ± 1.15	4.33 ± 1.76	10.33 ± 0.67
PTU	4.00 ± 1.29	6.00 ± 0.91	10.00 ± 2.04
Malathion	4.80 ± 0.97	5.20 ± 1.02	10.00 ± 1.26
Thyroxine	4.75 ± 0.95	5.50 ± 1.19	10.25 ± 0.25

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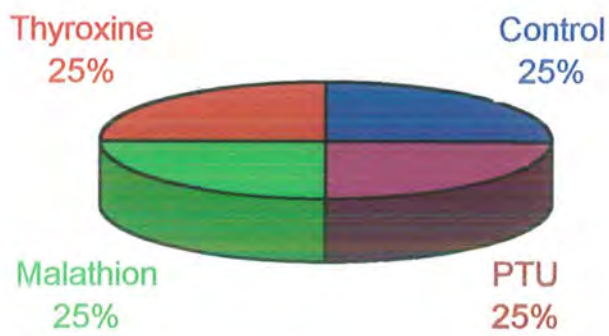
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Figure 1: Litter size (%) of control, PTU, malathion and thyroxine treated mothers **(A)** mothers receiving treatment from conception. to parturition **(B)** mothers receiving treatment from 0-25 day postpartum.

Pre- and postnatal malathion and thyroxine treated pups are normal in their behaviour, growth and physical development as compared to control.

CONTROL AND TREATED MOTHERS (FROM CONCEPTION TO 25 DAYS POSTPARTUM):

FEED CONSUMPTION AND WATER UPTAKE:

Weekly mean feed consumption of control, PTU, malathion and thyroxine treated mothers are shown in Table 2, 3; Figure 2A. There is no significant (P>0.05) difference in total mean feed consumption of the control, PTU, malathion and thyroxine treated mothers.

Weekly mean water uptake of control, PTU, malathion and thyroxine treated mothers are shown in Table 4, 5; Figure 2B. Malathion treated mothers (conception to parturition) show significant increase (P<0.01) in total mean water uptake as compared to control. PTU and thyroxine treated mothers show no difference (P>0.05) in total mean water uptake as compared to control.

BODY WEIGHT:

Weekly mean body weight of control, PTU, malathion and thyroxine treated mothers are shown in Table 6 and 7; Figure 3. Malathion treated mothers show a highly significant (P<0.01) increase in total mean body weight as compared to control. There is no significant (P>0.05) difference in total mean body weight of control, PTU and thyroxine treated mothers.

TABLE 2: WEEKLY FEED CONSUMPTION (g) OF CONTROL AND TREATED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM CONCEPTION TO PARTURITION.

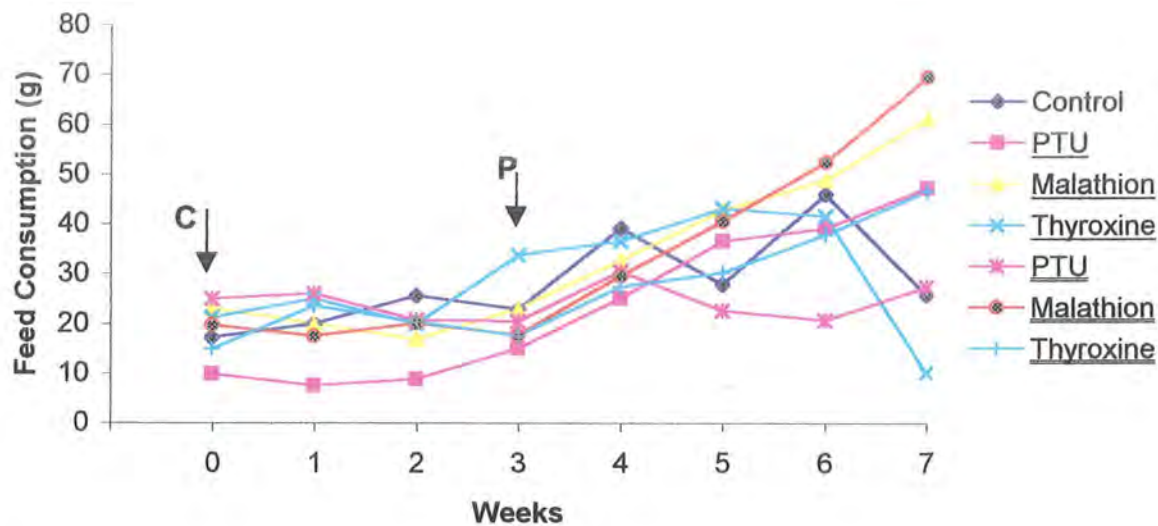| WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	17.04 ± 2.27	9.69 ± 3.43	22.87 ± 3.14	21.10 ± 2.85
1	19.75 ± 0.74	7.34 ± 1.15	19.80 ± 2.34	24.90 ± 2.56
2	25.52 ± 0.95	8.68 ± 0.75	16.66 ± 1.61	19.78 ± 2.39
3	22.74 ± 3.14	14.92 ± 3.43	22.93 ± 2.57	33.59 ± 3.83
4	39.12 ± 4.00	25.04 ± 1.05	32.59 ± 2.99	36.47 ± 5.97
5	27.86 ± 3.76	36.55 ± 1.64	42.70 ± 4.62	43.10 ± 4.47
6	45.91 ± 11.68	39.08 ± 2.32	48.81 ± 4.39	41.50 ± 8.60
7	25.78 ± 13.36	47.23 ± 8.21	61.26 ± 12.74	10.16 ± 1.24
Total Mean	27.97 ± 3.46	23.57 ± 5.55	33.45 ± 5.65	28.83 ± 4.12

Values (Mean ± SEM)
There is no significant (P>0.05) difference in mean feed consumption of control and treated mothers.

TABLE 3: WEEKLY FEED CONSUMPTION (g) OF CONTROL AND TREATED MOTHERS
RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM PARTURITION
TO 25 DAYS POSTPARTUM.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	17.04 ± 2.27	24.87 ± 4.15	19.53 ± 2.93	14.76 ± 1.70
1	19.75 ± 0.74	25.87 ± 4.45	17.34 ± 2.30	23.47 ± 6.52
2	25.52 ± 0.95	20.56 ± 2.84	19.98 ± 0.90	20.17 ± 0.63
3	22.74 ± 3.14	20.27 ± 3.91	17.64 ± 1.81	17.44 ± 1.60
4	39.12 ± 4.00	30.38 ± 6.42	29.48 ± 3.86	27.30 ± 3.98
5	27.86 ± 3.76	22.48 ± 6.32	40.57 ± 2.13	30.15 ± 4.86
6	45.91 ± 11.68	20.55 ± 3.81	52.45 ± 5.23	37.77 ± 10.70
7	25.78 ± 13.36	27.31 ± 6.52	69.51 ± 10.71	46.56 ± 16.57
Total Mean	27.97 ± 3.46	24.04 ± 1.31	33.31 ± 6.83	27.20 ± 3.80

Values (Mean ± SEM)
There is no significant (P>0.05) difference in mean feed consumption of control and
treated mothers.

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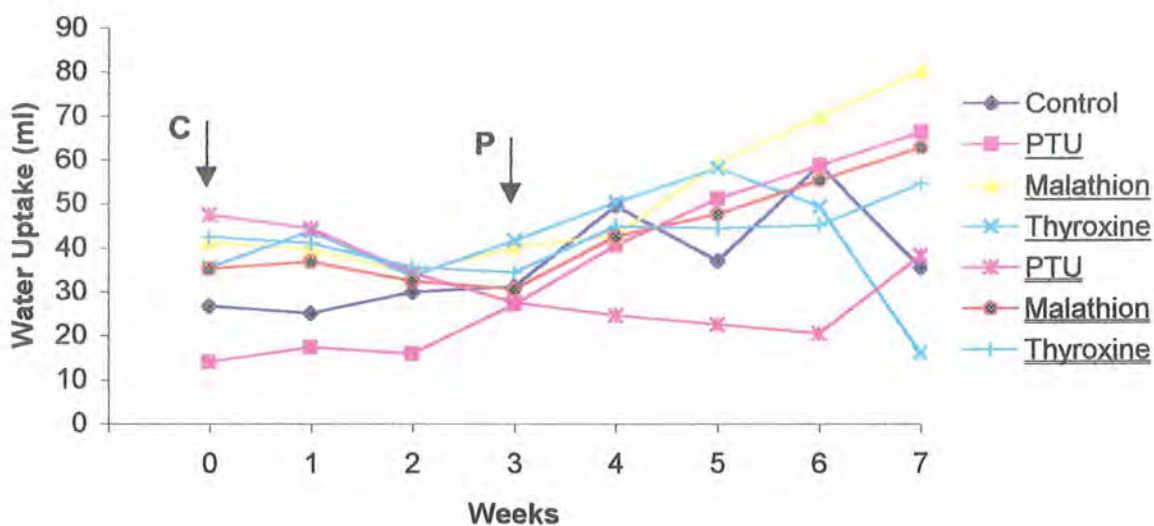
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Figure 2: Weekly mean feed consumption **(A)** mean water uptake **(B)** of control, PTU, malathion and thyroxine treated mothers.Single line indicates mothers receiving treatment from conception to parturition.Double line indicates mothers receiving treatment from 0-25 day postpartum. C=Conception and P=Parturiton.

TABLE 4: WEEKLY WATER UPTAKE (ml) OF CONTROL AND TREATED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM CONCEPTION TO PARTURITION.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	26.63 ± 4.51	13.90 ± 1.65	41.17 ± 3.43	35.34 ± 4.85
1	24.98 ± 3.07	17.30 ± 0.90	39.47 ± 3.54	43.80 ± 3.60
2	29.87 ± 3.25	15.83 ± 0.75	34.23 ± 3.82	33.54 ± 3.93
3	31.11 ± 2.56	27.10 ± 2.94	40.13 ± 8.28	41.68 ± 4.21
4	49.58 ± 1.37	40.53 ± 4.49	42.90 ± 3.99	50.36 ± 7.74
5	37.03 ± 5.90	51.17 ± 3.26	59.20 ± 5.77	58.30 ± 6.73
6	59.00 ± 1.05	58.83 ± 3.38	69.93 ± 6.37	49.48 ± 8.99
7	35.53 ± 14.29	66.37 ± 4.43	80.49 ± 18.39	16.05 ± 2.31
Total Mean	36.72 ± 4.19	36.38 ± 7.34	50.94 ± 5.96^{a**}	41.07 ± 4.59

Values (Mean ± SEM)
a= treated groups vs control
P<0.01**

TABLE 5: WEEKLY WATER UPTAKE (ml) OF CONTROL AND TREATED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM PARTURITION TO 25 DAYS POSTPARTUM.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	26.63 ± 4.51	47.38 ± 6.55	35.13 ± 5.44	42.51 ± 3.58
1	24.98 ± 3.07	44.38 ± 2.68	36.70 ± 2.26	41.01 ± 5.26
2	29.87 ± 3.25	34.20 ± 1.98	32.30 ± 2.18	35.61 ± 1.52
3	31.11 ± 2.56	27.52 ± 5.54	30.60 ± 4.03	34.43 ± 2.65
4	49.58 ± 1.37	24.61 ± 3.02	42.63 ± 4.50	44.86 ± 5.29
5	37.03 ± 5.90	22.56 ± 3.95	47.70 ± 4.54	44.46 ± 6.77
6	59.00 ± 1.05	20.49 ± 5.74	55.43 ± 4.42	45.29 ± 11.33
7	35.53 ± 14.29	38.38 ± 5.94	62.89 ± 15.08	54.71 ± 13.54
Total Mean	36.72 ± 4.19	32.44 ± 3.61	42.92 ± 4.10	42.86 ± 2.24

Values (Mean ± SEM)
There is no significant (P>0.05) difference in mean water uptake of control and treated mothers.
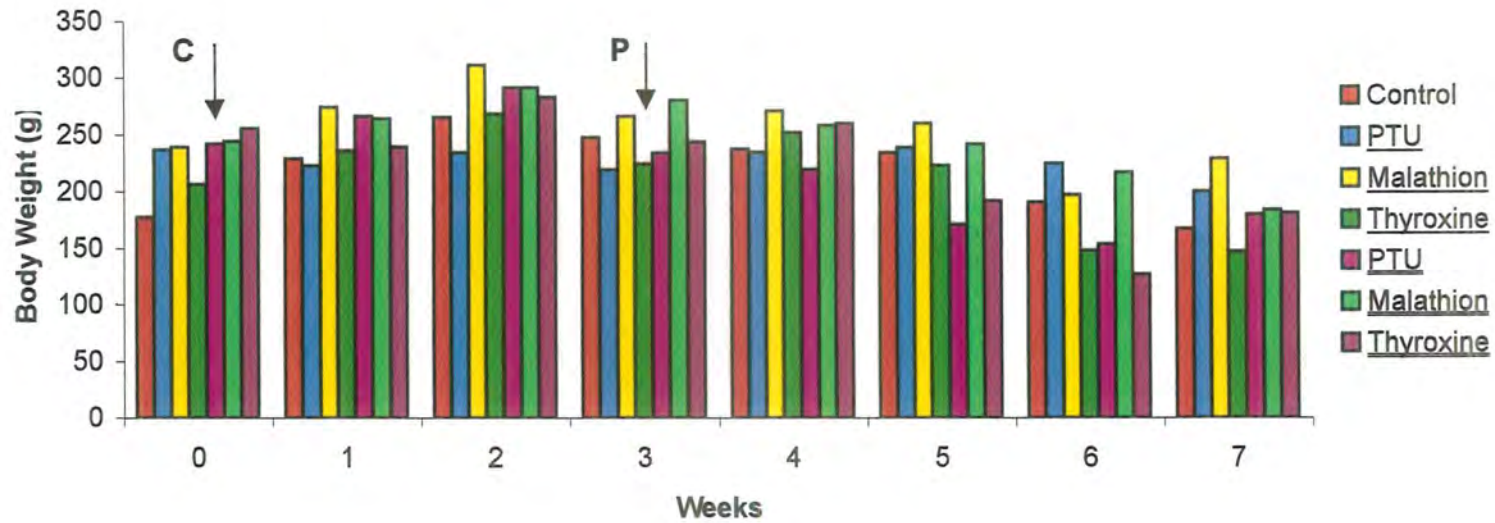
Figure 3: Weekly mean body weights of control, pre- and postnatal PTU, malathion and thyroxine treated mothers. Single line indicates mothers receiving treatment from conception to parturition. Double line indicates mother receiving treatment from 0-25 day postpartum. **C**=conception and **P**=Parturiton.

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TABLE 6: WEEKLY BODY WEIGHT(g) OF CONTROL AND TREARED MOTHERS RECEIVING PTU, MALATHION AND THYROXINE TREATMENT FROM CONCEPTION TO PARTURITION.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	177.62 ± 18.68	237.00 ± 7.18	239.00 ± 5.79	206.53 ± 10.63
1	229.22 ± 20.97	223.00 ± 11.25	274.00 ± 6.96	235.79 ± 13.17
2	264.96 ± 19.02	234.00 ± 10.77	311.00 ± 7.97	268.04 ± 18.17
3	246.97 ± 12.56	219.00 ± 10.05	266.00 ± 7.81	223.63 ± 28.22
4	236.64 ± 25.21	234.00 ± 7.81	270.00 ± 7.07	250.88 ± 2.40
5	233.32 ± 9.87	238.00 ± 13.47	259.00 ± 8.12	222.05 ± 11.31
6	190.08 ± 13.82	224.00 ± 10.42	196.36 ± 31.09	147.16 ± 11.60
7	166.47 ± 1.31	199.31 ± 0.64	227.86 ± 1.48	145.86 ± 1.54
Total Mean	218.20 ± 12.55	226.00 ± 4.57	255.40 ± 12.17[a**]	212.50 ± 15.85

Values (Mean ± SEM)
a= treated groups vs control
P<0.01**

TABLE 7: WEEKLY BODY WEIGHT (g) OF CONTROL AND TREATED MOTHERS
RECEIVING PTU, MALATHION AND THYROXINE FROM PARTURITION
TO 25 DAYS POSTPARTUM.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
0	177.62 ± 18.68	241.77 ± 17.71	244.00 ± 6.20	255.40 ± 12.05
1	229.22 ± 20.97	266.41 ± 18.35	264.00 ± 10.05	239.13 ± 15.40
2	264.96 ± 19.02	291.29 ± 18.02	291.00 ± 12.39	282.62 ± 10.19
3	246.97 ± 12.56	233.28 ± 13.94	280.00 ± 27.20	243.10 ± 28.40
4	236.64 ± 25.21	219.02 ± 6.39	257.00 ± 7.52	259.08 ± 11.04
5	233.32 ± 9.87	170.45 ± 38.03	241.00 ± 6.40	191.06 ± 43.45
6	190.08 ± 13.82	152.60 ± 37.56	216.00 ± 11.77	126.06 ± 45.03
7	166.47 ± 1.31	178.67 ± 18.87	182.57 ± 5.91	180.05 ± 5.81
Total Mean	218.20 ± 12.55	219.20 ± 17.21	246.90 ± 12.37[a**]	222.10 ± 18.33

Values (Mean ± SEM)
a= treated groups vs control
P<0.01**

CONTROL, PRE- AND POSTNATAL TREATED YOUNG FEMALE RATS:

FEED AND WATER CONSUMPTION (FROM WEANING UPTO 120 DAYS):

Weekly mean feed consumption of control, pre- and postnatal PTU, malathion and thyroxine treated young female rats are shown in Table 8 and 9; Figure 4A. Pre- and postnatal PTU and malathion show a significant (P<0.01) reduction in total mean feed consumption as compared to control. Prenatal thyroxine treatment significantly (P<0.001) increases ,and postnatal thyroxine treatment significantly (P<0.001) decreases the total mean feed consumption as compared to control.

Weekly mean water uptake of control, pre- and postnatal PTU, malathion and thyroxine treated young female rats are shown in Table 10 and 11; Figure 4B. Total mean water uptake of pre- and postnatal PTU and malathion treatment show significant (P<0.01) decrease as compared to control. In comparison to control prenatal thyroxine treatment exhibit highly significant (P<0.001) increase, while postnatal thyroxine treatment show highly significant (P<0.001) decrease in total mean water uptake.

BODY WEIGHT (FROM BIRTH UPTO 120 DAYS):

Total mean body weight of control, pre- and postnatal PTU, malathion and thyroxine treated young female rats are shown in Table 12 and their weekly mean body weight are shown in Figure 5. Total mean body weight of prenatal PTU treated female rats (155 ± 21.57g) exhibit highly significant (P<0.0001) increase
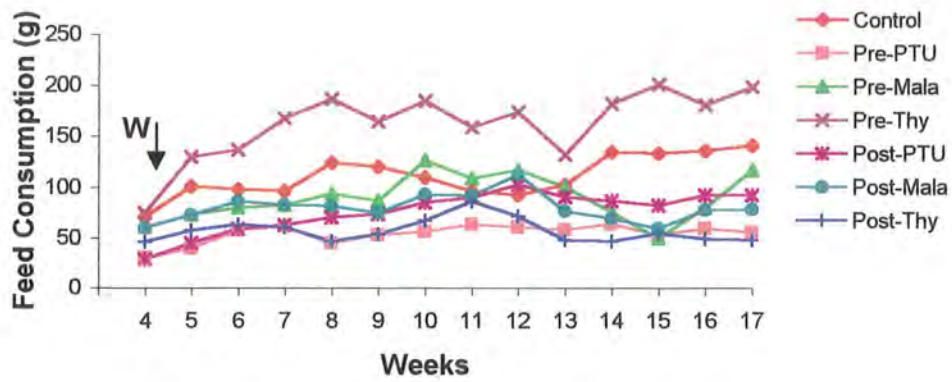
TABLE 8: WEEKLY FEED CONSUMPTION (g) OF CONTROL AND PRENATAL PTU, MALATHION AND THYROXINE TREATED FEMALE RATS FROM WEANING TO 120 DAYS OF AGE.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
4	71.00 ± 8.50	30.20 ± 5.67	60.64 ± 12.04	74.57 ± 11.89
5	100.30 ± 18.37	39.76 ± 6.61	72.83 ± 15.33	129.16 ± 17.24
6	98.08 ± 17.18	57.54 ± 13.05	79.73 ± 16.36	136.41 ± 22.97
7	95.97 ± 15.29	61.26 ± 11.54	82.34 ± 18.76	167.24 ± 32.57
8	123.82 ± 18.15	44.81 ± 9.05	93.38 ± 25.65	186.23 ± 39.71
9	119.89 ± 14.49	53.05 ± 12.66	86.74 ± 17.47	164.02 ± 38.98
10	108.96 ± 10.06	56.41 ± 9.84	126.41 ± 35.11	184.45 ± 34.57
11	96.00 ± 6.39	62.93 ± 15.53	108.40 ± 25.41	158.25 ± 27.08
12	92.04 ± 9.78	60.87 ± 13.77	117.01 ± 36.59	173.50 ± 25.85
13	102.58 ± 20.31	58.30 ± 12.80	100.61 ± 29.87	131.78 ± 6.34
14	134.70 ± 26.98	63.60 ± 15.01	75.93 ± 10.77	181.79 ± 30.74
15	133.05 ± 19.60	53.29 ± 11.55	49.86 ± 14.09	200.60 ± 33.60
16	135.53 ± 14.31	59.13 ± 11.11	79.82 ± 9.50	180.27 ± 20.10
17	140.58 ± 6.12	55.81 ± 9.97	116.81 ± 20.89	198.30 ± 26.86
Total Mean	110.90 ± 5.52	54.07 ± 2.56[a***]	89.32 ± 5.96[a***]	161.90 ± 9.11

Values(Mean ± SEM)
a= treated groups vs control

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TABLE 9: WEEKLY FEED CONSUMPTION(g) OF CONTROL AND POSTNATAL PTU, MALATHION AND THYROXINE TREATED FEMALE RATS FROM WEANING TO 120 DAYS OF AGE.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
4	71.00 ± 8.50	29.16 ± 16.19	59.88 ± 15.55	46.36 ± 13.83
5	100.30 ± 18.37	44.73 ± 17.30	72.73 ± 18.10	57.17 ± 15.09
6	98.08 ± 17.18	58.49 ± 19.89	85.56 ± 20.64	63.40 ± 17.36
7	95.97 ± 15.29	62.53 ± 19.84	82.92 ± 23.55	60.54 ± 17.02
8	123.82 ± 18.15	70.07 ± 16.47	81.39 ± 21.64	46.86 ± 13.05
9	119.89 ± 14.49	72.94 ± 14.25	74.24 ± 17.29	53.84 ± 12.25
10	108.96 ± 10.06	84.72 ± 19.73	92.68 ± 26.08	67.20 ± 15.99
11	96.00 ± 6.39	89.37 ± 18.97	91.58 ± 25.97	86.14 ± 23.14
12	92.04 ± 9.78	101.97 ± 28.85	112.29 ± 37.85	71.43 ± 18.18
13	102.58 ± 20.31	90.35 ± 17.56	76.12 ± 19.03	47.95 ± 13.12
14	134.70 ± 26.98	86.22 ± 14.36	69.27 ± 15.65	46.90 ± 10.01
15	133.05 ± 19.60	81.88 ± 15.57	59.64 ± 11.78	54.24 ± 12.05
16	135.53 ± 14.31	92.40 ± 16.46	77.56 ± 16.16	49.49 ± 10.11
17	140.58 ± 6.12	92.45 ± 16.84	78.10 ± 17.56	47.71 ± 7.77
Total Mear	110.90 ± 5.52	75.52 ± 5.50[a]***	79.57 ± 3.64[a]***	57.09 ± 3.12[a]***

Values(Mean ± SEM)
a= treated groups vs control
P<0.001***

A
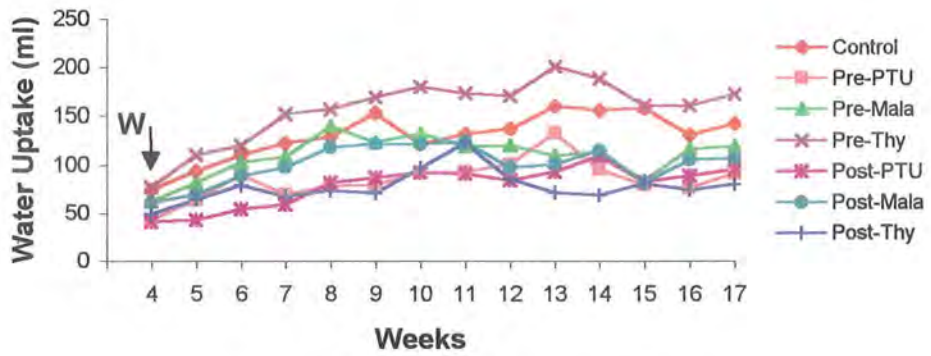
B

Figure 4: Weekly mean feed consumption(A) mean water uptake(B) of control, prenatal and postnatal PTU, malathion and thyroxine treated young female rats. W=Weaning.

TABLE 10: WEEKLY WATER UPTAKE (ml) OF CONTROL AND PRENATAL PTU, MALATHION AND THYROXINE TREATED FEMALE RATS FROM WEANING TO 120 DAYS OF AGE.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
4	73.57 ± 5.69	42.23 ± 7.03	61.97 ± 13.12	76.13 ± 9.67
5	92.77 ± 9.79	63.07 ± 12.12	81.43 ± 20.66	109.53 ± 6.56
6	109.50 ± 9.13	89.87 ± 24.13	102.37 ± 31.38	119.67 ± 14.20
7	122.43 ± 3.90	69.20 ± 11.29	108.03 ± 28.74	151.27 ± 16.45
8	128.17 ± 2.34	77.70 ± 12.24	139.37 ± 39.12	156.17 ± 13.72
9	152.93 ± 8.16	79.23 ± 17.65	123.00 ± 33.20	168.70 ± 15.93
10	120.97 ± 8.06	91.50 ± 27.30	132.43 ± 36.12	179.47 ± 17.05
11	131.37 ± 9.62	92.67 ± 33.34	119.13 ± 30.12	172.83 ± 13.52
12	137.03 ± 10.27	100.53 ± 23.65	119.67 ± 30.52	170.47 ± 16.87
13	159.35 ± 5.44	132.68 ± 47.45	109.10 ± 27.05	200.85 ± 15.99
14	155.30 ± 2.86	95.15 ± 26.72	113.50 ± 33.45	188.20 ± 25.84
15	157.45 ± 5.34	79.23 ± 19.37	83.03 ± 19.39	160.17 ± 12.87
16	131.27 ± 10.21	75.57 ± 17.16	116.33 ± 35.18	160.17 ± 14.24
17	142.41 ± 6.71	90.77 ± 21.69	119.03 ± 37.43	172.37 ± 17.76
Total Mean	129.60 ± 6.68	84.24 ± 5.51^{a**}	109.20 ± 5.62^{a**}	156.10 ± 8.90^{a***}

Values(Mean ± SEM)
a= treated groups vs control
$P<0.01$**
$P<0.001$***

TABLE 11: WEEKLY WATER UPTAKE (ml) OF CONTROL AND POSTNATAL PTU,
MALATHION AND THYROXINE TREATED FEMALE RATS FROM WEANING
TO 120 DAYS OF AGE.

WEEKS	CONTROL	PTU	MALATHION	THYROXINE
4	73.57 ± 5.69	40.26 ± 10.74	60.67 ± 11.46	48.75 ± 11.47
5	92.77 ± 9.79	42.83 ± 13.40	68.83 ± 13.78	63.75 ± 13.82
6	109.50 ± 9.13	53.93 ± 19.52	88.13 ± 17.21	78.21 ± 20.59
7	122.43 ± 3.90	58.77 ± 20.40	97.33 ± 18.06	67.04 ± 21.09
8	128.17 ± 2.34	80.90 ± 23.34	117.40 ± 24.78	72.88 ± 17.68
9	152.93 ± 8.16	86.53 ± 28.07	121.43 ± 34.22	70.67 ± 21.61
10	120.97 ± 8.06	91.67 ± 25.29	120.87 ± 29.98	96.50 ± 16.13
11	131.37 ± 9.62	90.33 ± 26.44	123.73 ± 29.80	122.54 ± 10.90
12	137.03 ± 10.27	83.93 ± 18.31	96.87 ± 21.62	85.17 ± 12.08
13	159.35 ± 5.44	92.53 ± 23.20	100.67 ± 32.65	71.17 ± 17.96
14	155.30 ± 2.86	109.13 ± 27.08	115.20 ± 35.96	68.38 ± 15.04
15	157.45 ± 5.34	82.40 ± 15.83	81.63 ± 12.91	80.19 ± 16.95
16	131.27 ± 10.21	88.70 ± 23.12	105.33 ± 32.20	73.83 ± 18.04
17	142.41 ± 6.71	94.93 ± 23.82	107.37 ± 34.34	79.92 ± 18.33
Total Mean	129.60 ± 6.68	78.35 ± 5.57^{a**}	100.40 ± 5.28^{a**}	77.07 ± 4.56^{a***}

Values(Mean ± SEM)
a= treated groups vs control
$P < 0.01**$
$P < 0.001***$

TABLE 12: MEAN BODY WEIGHT OF CONTROL, PRE- AND POSTNATAL PTU, MALATHION AND THYROXINE TREATED YOUNG FEMALE RATS FROM BIRTH TO 120 DAYS OF AGE.

Groups n=10	Mean Body weight (g) Mean ± SEM
Control	136.10 ± 20.12
Prenatal Treatment	
PTU	155.20 ± 21.57^{a****}
Malathion	136.60 ± 20.34
Thyroxine	126.10 ± 18.94^{a***}
Postnatal Treatment	
PTU	109.10 ± 17.97^{a****}
Malathion	143.30 ± 20.79
Thyroxine	102.10 ± 18.45^{a***}

Values(Mean ± SEM)
a= treated groups vs control
$P<0.001$***
$P<0.0001$****
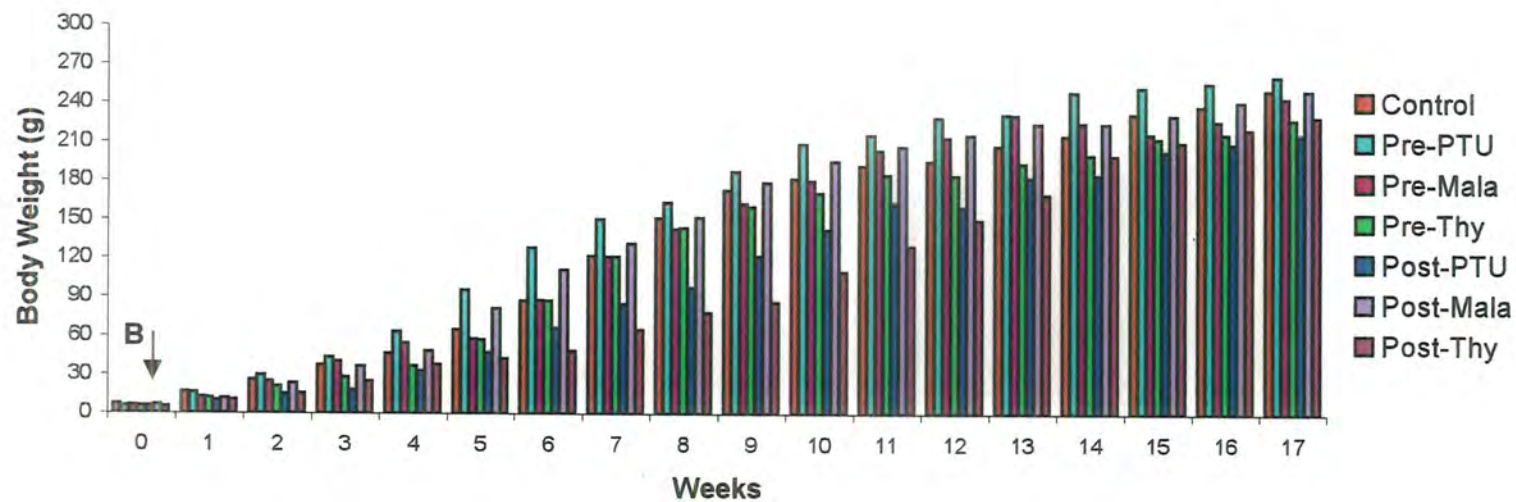
Figure 5: Weekly mean body weight (0-120 days) of control, pre- and postnatal PTU, malathion and thyroxine treated young female rats. **B**=Birthday.

compared to control (136 ± 20.12g), where as postnatal PTU treated female rats (109 ± 17.97g) show a highly significant (P<0.0001) decrease in total mean body weight as compared to control. There is no significant (P>0.05) difference in total mean body weight of female rats of control, pre- and postnatal malathion treatment group. Total mean body weight of prenatal and postnatal thyroxine treated female rats (126.10 ± 18.94, 102.10 ± 18.45g) exhibit highly significant (P<0.001) reduction as compared to control.

MORPHOLOGICAL STUDIES:

OVARIAN WEIGHT:

Paired ovarian weight of control and treated groups are shown in Table 13; Figure 6. Paired ovarian weight of prenatal PTU, malathion and thyroxine treatment groups although vary from control, but this variation is not appreciable (P>0.05).

Paired ovarian weight of postnatal PTU treatment group (54.30 ± 3.62 mg) exhibit highly significant (P<0.001) reduction as compared to paired ovarian weight of control (73.50 ± 2.67 mg) and prenatal PTU treatment group (82.50 ± 3.31 mg).

Paired ovarian weight of postnatal malathion treatment group (68.10 ± 1.94 mg) is significantly (P<0.01) lower than paired ovarian weight of prenatal malathion treatment group (85.20 ± 4.43 mg), while it exhibit small but non significant (P>0.05) difference to control.
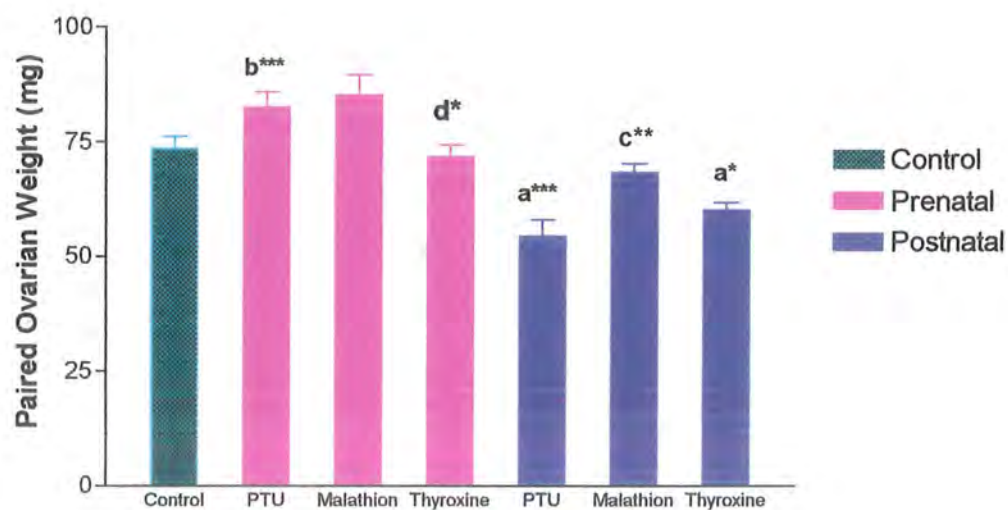
Figure 6: Ovarian weight of control and treated female rats at 120 day of age. Data are presented as Mean ± SEM. Postnatal PTU and thyroxine treatment group show significant reduction ***P<0.001and *P<0.05 respectively in ovarian weight compared to control a). Postnatal PTU indicate significant reduction ***P<0.001 than prenatal PTU treated group b). Postnatal thyroxine show significant reduction *P<0.05 than prenatal thyroxine treaed group d). Postnatal malathion show significant reduction **P<0.01 than prenatal malathion treated group c).

TABLE 13: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT DURING PRE- AND POSTNATAL PERIOD ON OVARIAN WEIGHT AND DIAMETER OF FEMALE RAT AT 120 DAYS OF AGE.

Groups n=10	Total Ovarian Weight (mg)	Right Ovarian Diameter (mm)	Left Ovarian Diameter (mm)
Control	73.50 ± 2.67	6.12 ± 0.15	6.24 ± 0.19
Prenatal Treatment			
PTU	82.50 ± 3.31[b***]	5.93 ± 0.13	5.89 ± 0.16
Malathion	85.20 ± 4.43	6.01 ± 0.18	6.07 ± 0.20
Thyroxine	71.60 ± 2.84[d*]	5.95 ± 0.11	6.19 ± 0.15
Postnatal Treatment			
PTU	54.30 ± 3.62[a***]	5.44 ± 0.22	5.19 ± 0.16
Malathion	68.10 ± 1.94[c**]	5.65 ± 0.28	5.67 ± 0.17
Thyroxine	60.10 ± 1.71[a*]	5.54 ± 0.18	5.61 ± 0.15

Values (Mean ± SEM)
a= treated groups vs control.
b= prenatal vs postnatal PTU treatment group.
c= prenatal vs postnatal malathion treatment group.
d= prenatal vs postnatal thyroxine treatment group
P<0.05* and P<0.001***
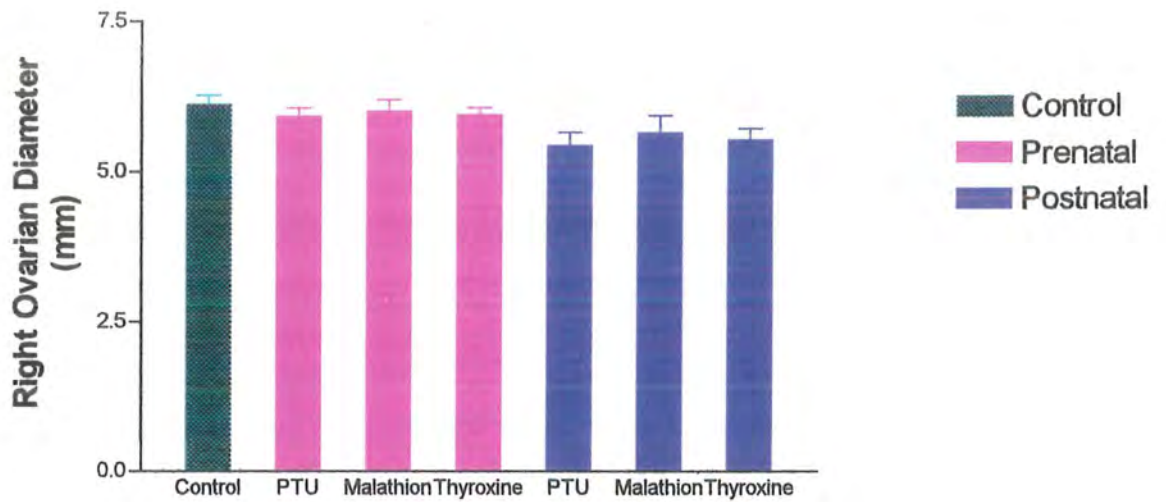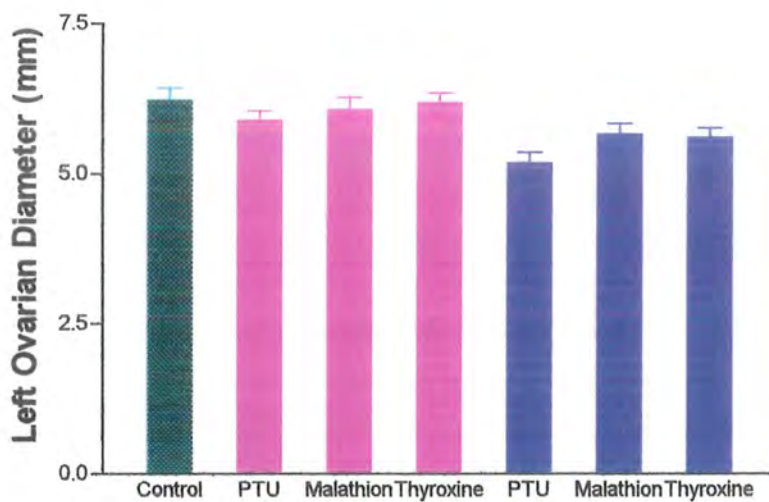
Figure 7: Ovarian diameter of female rat at 120 day of age. Data are expressed as Mean ± SEM. Right **(A)**and left **(B)** ovarian diameter shows non significant (P>0.05) difference between control, pre- and postnatal treatment groups.

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Paired ovarian weight of thyroxine treatment (60.10 ± 1.71 mg) during postnatal period exhibit significant (P<0.05) reduction than control.

OVARIAN DIAMETER :

Ovarian diameter of control, pre- and postnatal PTU, malathion and thyroxine treatment groups are shown in Table 13; Figure 7A and 7B. Right and left ovarian diameter of PTU, malathion and thyroxine treatment groups although vary from control, but the difference in all the groups is non significant (P>0.05).

HISTOLOGICAL OBSERVATIONS:

Ovary in rats is a paired structure, which is situated in abdominal cavity. It is observed that the outer most layer which surrounds the ovary is surface epithelium. Beneath it lies the tunica albuginea. Immediately beneath the tunica albuginea there is a cortical region which contain numerous follicles at various developmental stages, embedded in the ovarian stroma. Many blood vessels have also been observed in the stroma (Figure 8A and 8B). Among the follicles most common are the primary follicles which are smallest and simpler in structure. In primary follicles it is observed that oocyte is surrounded by a layer of granulosa cells. Nucleus which lies in the centre of oocyte is also observed. No difference is found in the structure of primary follicle of control, pre- and postnatal PTU, malahtion and thyroxine treated rats at 120 day of age (Figure 9A and 9B).
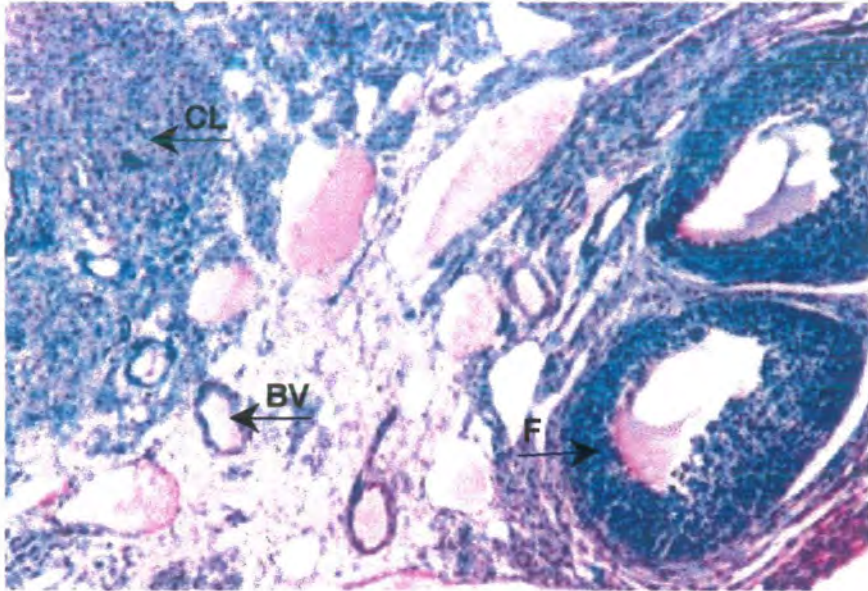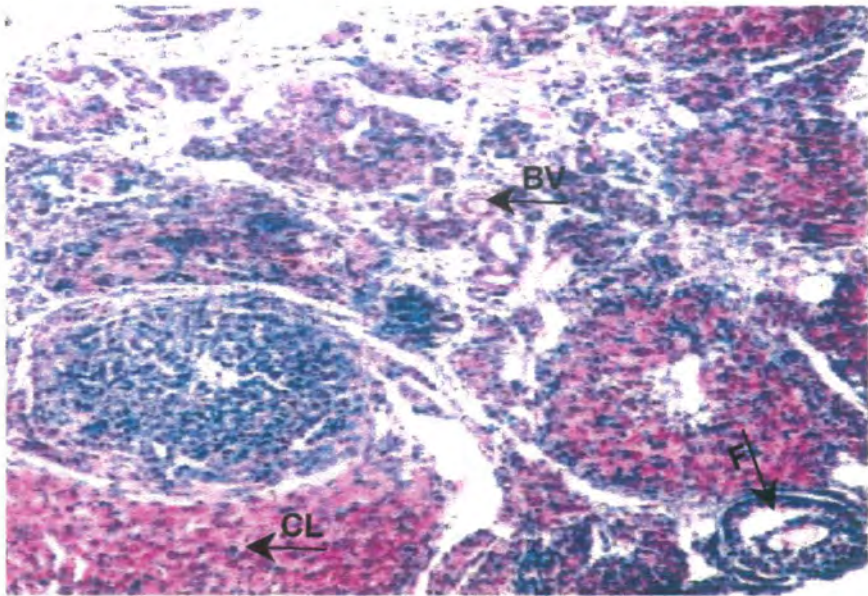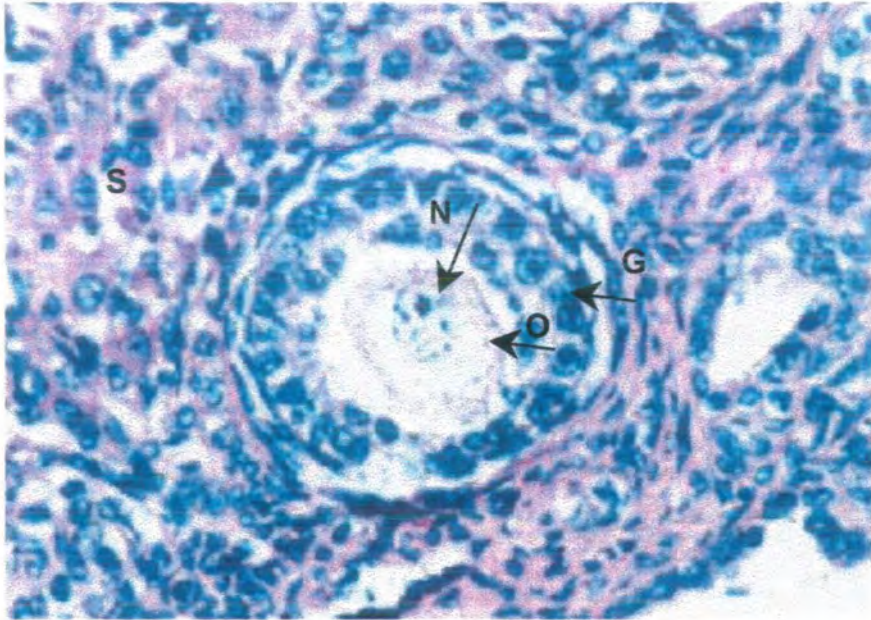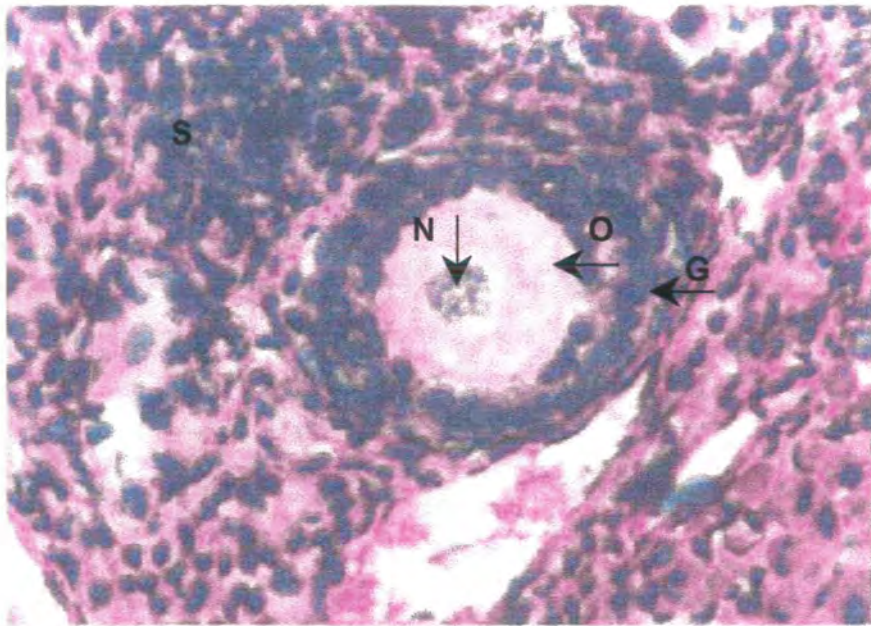
Figure 8: Photomicrograph of the rat ovary at 120 days of age showing stroma containing follicle (F) corpus luteum (CL) blood vessel (BV). No difference is found in control and all treated groups. Control **(A)** postnatal thyroxine treatment group **(B) (H.E)** × 102.6.
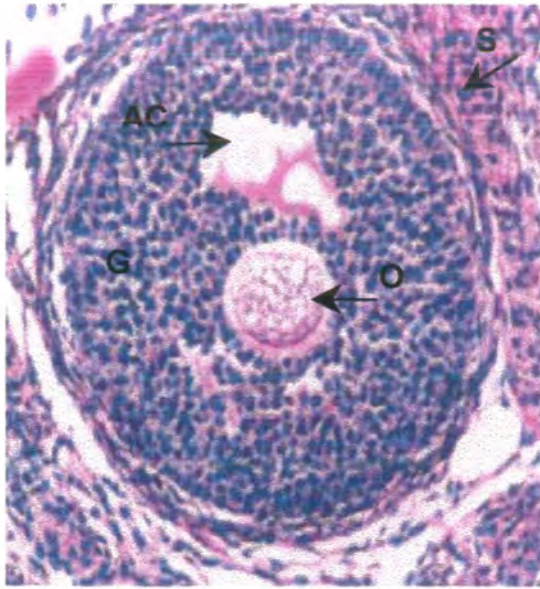
A

B

Figure 9: Photomicrograph of the rat ovary at 120 days of age showing Primary follicles. Nucleus (N) oocyte (O) granulosa cells (G) stroma (S). No difference is found in control and other treated groups. Control **(A)** postnatal PTU treatment group **(B)** at ×330 **H.E)**.

Developing follicles of different sizes are observed in the ovaries of control rats. In developing follicles the oocyte is surrounded by multilayered granulosa cells. It is observed that number of granulosa layers which contribute the size of follicles vary in different developing follicles (Figure 10A). No difference is found in developing follicles of control and all treated groups. Polyovular follicles are observed in one female of postnatal PTU (Figure 10B) and in two female of postnatal thyroxine treated rats (Figure 10C and 10D).Polyovular follicle means presence of more than one oocyte in the follicular envelop.

Largest follicles or the graafian follicles have also been observed in the ovaries of control and all treatment groups. A fluid filled antral cavity (antrum) is present in the graafian follicles. The size of antrum in developing follicles is variable, but fully formed large antrum is only observed in the graafian follicles. It is found that with in the antral cavity, oocyte is embedded in the mass of granulosa cells, called as crona radiata, lies at one side of the follicles, attached to the peripheral granulosa by means of arm like structure the cumulus oophorus (Figure 11A). No difference is found in the graafian follicle of control and treated groups (Figure 11B,11C and 11D).
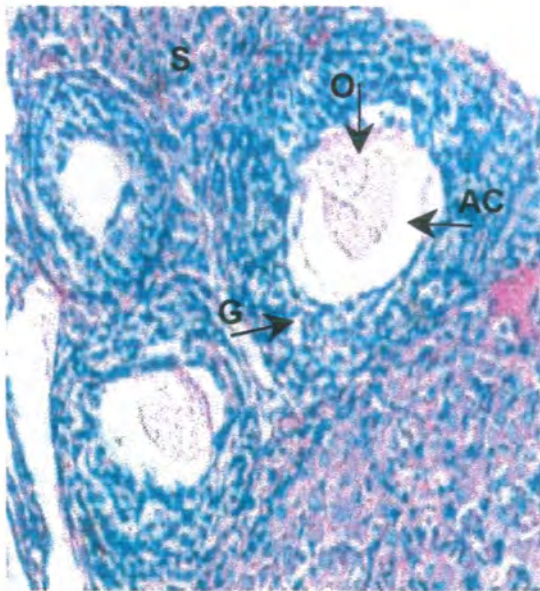
Antral cavity is surrounded by granulosa layers above which layers of theca cells have been observed (Figure 12A). There is no difference in granulosa and theca layers of control, pre- and postnatal PTU, malathion and thyroxine treatment group (Figure 12B and 12C).
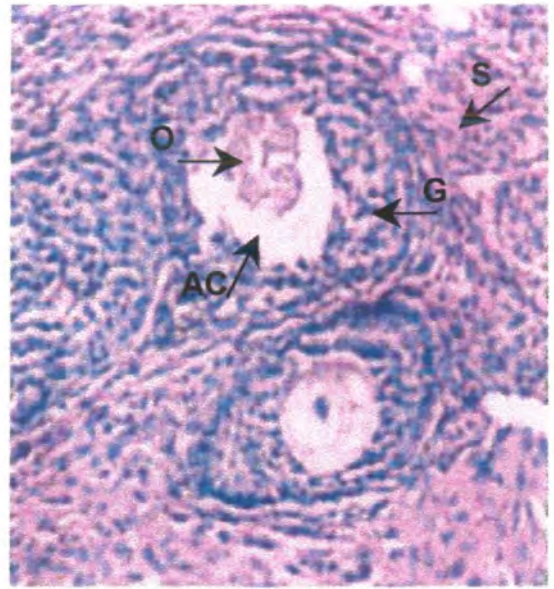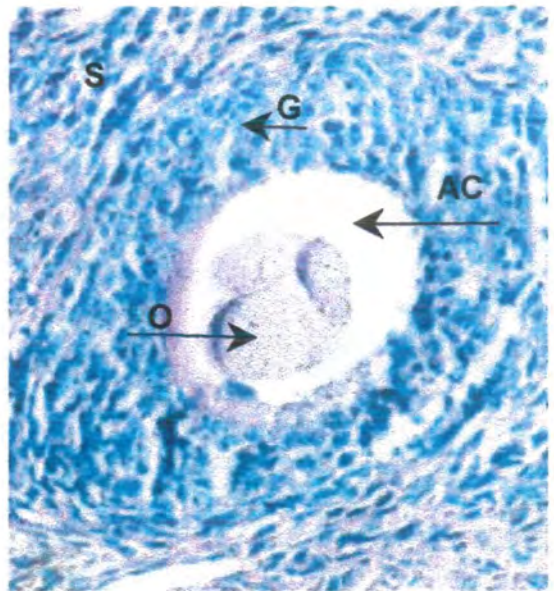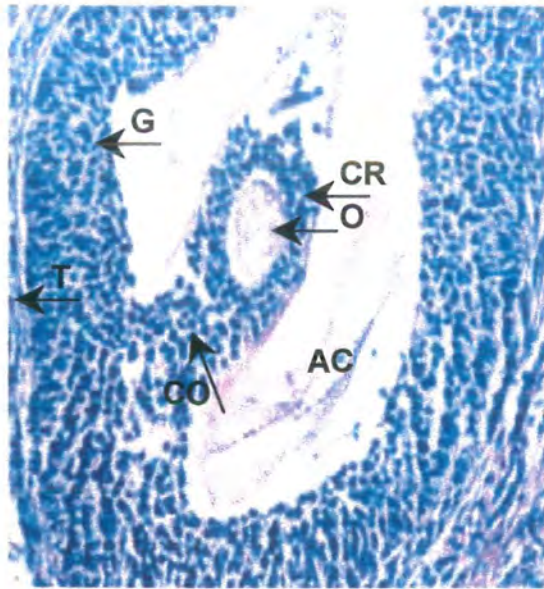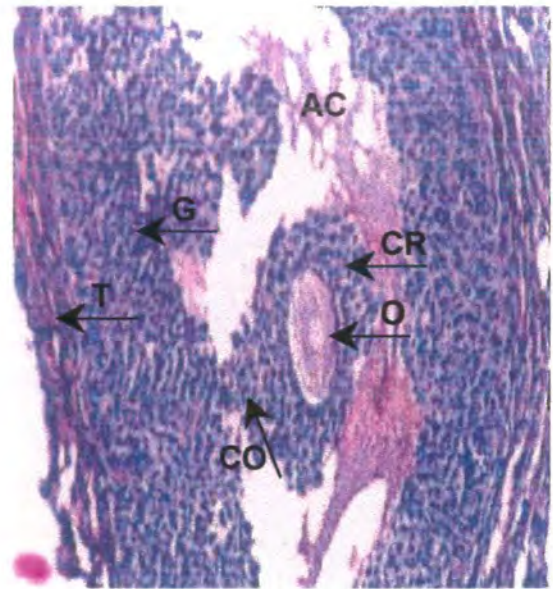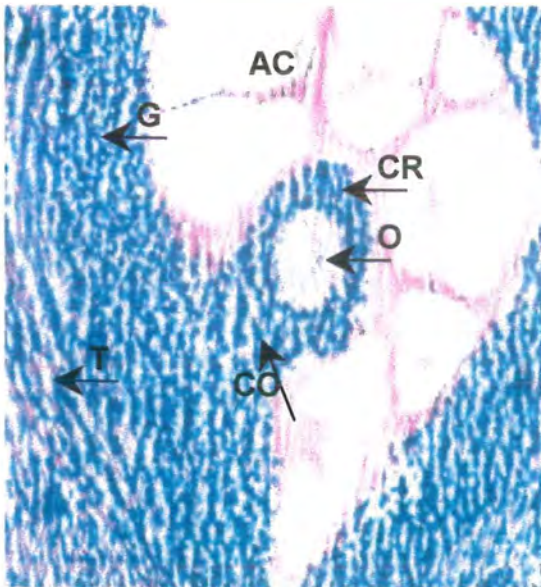
Figure 10: Photomicrograph of rat ovary at 120 days of age showing developing follicle in control **(A)**, polyovular follicles in postnatal PTU **(B)** postnatal thyroxine **(C, D)** treatment group. Granulosa cells (G) oocyte (O) antral cavity (AC) stroma (S) **(H.E)** × 228.
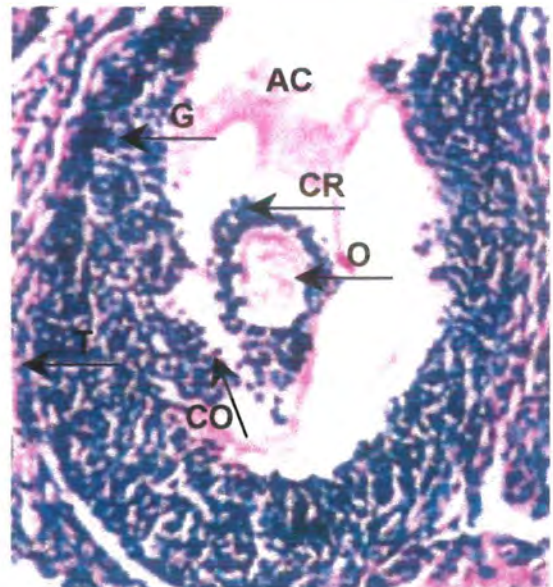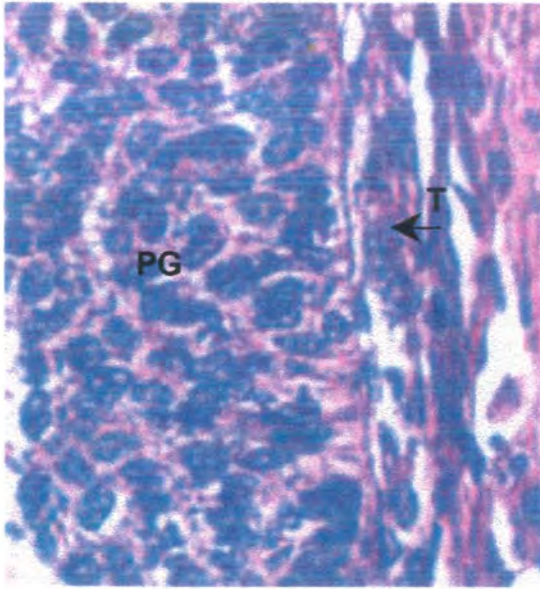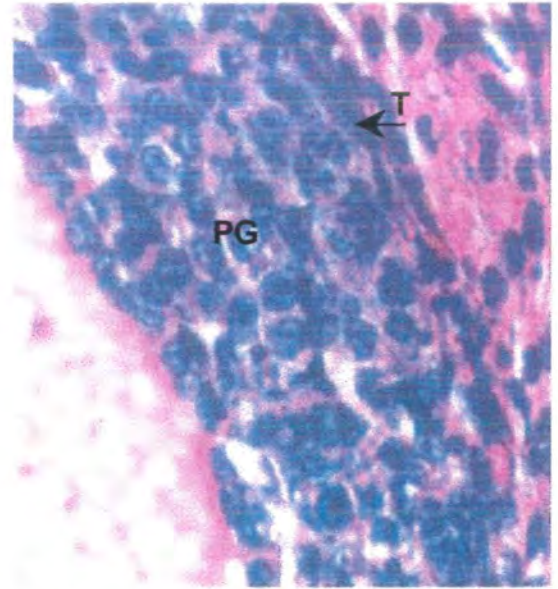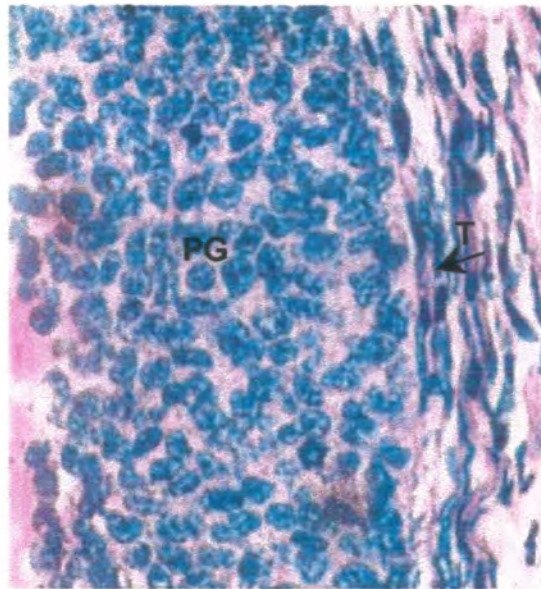
Figure 11: Photomicrograph of the rat ovary at 120 days of age showing graafian follicle containing oocyte (O) cumulus oophorus (CO) crona radiata (CR) antral cavity (AC) granulosa layer (G) theca layer (T). No difference is found in control and all treated groups.Control **(A)** postnatal thyroxine **(B)** postnatal PTU **(C)** prenatal PTU **(D)** treatment group **(H.E)** × 228.

Figure 12: Photomicrograph of the graafian follicles in rat ovary at 120 days of age showing peripheral granulosa layer (PG) and theca layer (T). No difference in control **(A)** postnatal PTU **(B)** and postnatal thyroxine **(C)** treatment group **(H.E)** × 416.

It is observed that corpus luteum of control rat ovary contains two types of cells, the larger one are the large luteal cells and the smaller one are the small luteal cells. These cells are found to be very close to one another and present in almost equal proportion. Large luteal cells have spherical shaped large nucleus while, small luteal cells have a spindle shaped nucleus (Figure 13A and 13B). It is noticed that corpus luteum of postnatal PTU and thyroxine treatment group have a remarkable increased number of large luteal cells and lesser number small luteal cells (Figure 13C, 13D and 13E). All other treated groups show no difference in quantity of large and small luteal cells of corpus luteum.

MORPHOMETRIC ANALYSIS:

DIAMETER OF GRAAFIAN FOLLICLES AND OOCYTE:

Total mean diameter of graafian follicles of control, pre- and postnatal PTU, malathion and thyroxine treated ovaries are shown in Table 14; Figure 14A. Diameter of graafian follicles of prenatal PTU treatment group (585.40±24.03 μm) exhibit highly significant (P<0.001) increase as compared to diameter of control (414.80±21.61 μm) and postnatal PTU treatment group (396.00±26.00 μm).

Pre- and postnatal malathion and thyroxine treatment groups does not show any significant (P>0.05) difference in comparison with control.

There is very slight but non significant (P>0.05) difference in diameter of oocyte of control, pre- and postnatal PTU, malathion and thyroxine treatment groups (Table 14).

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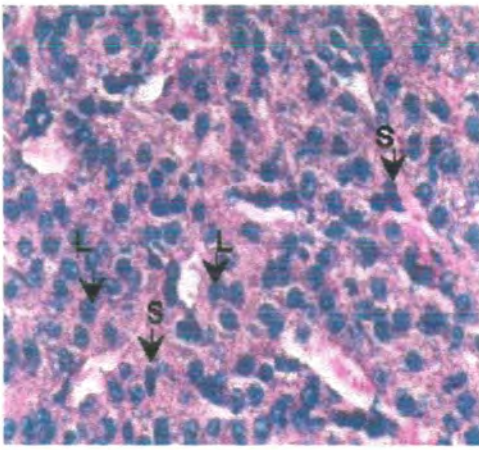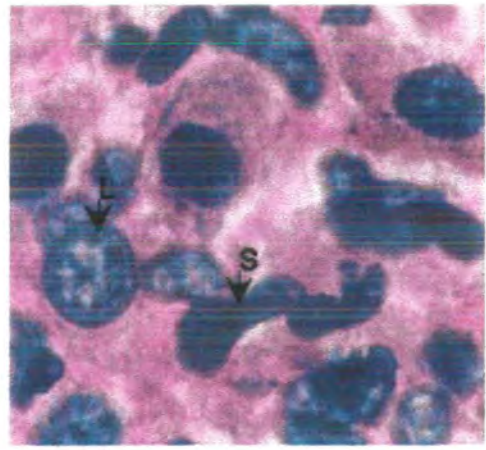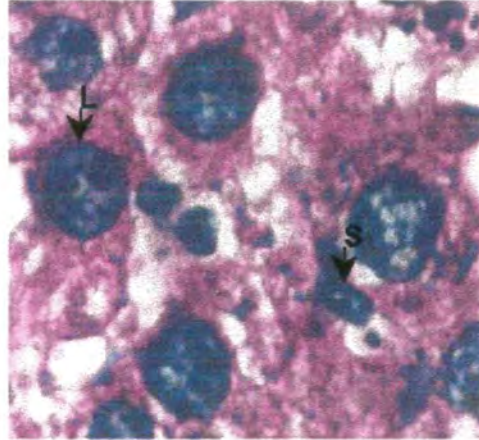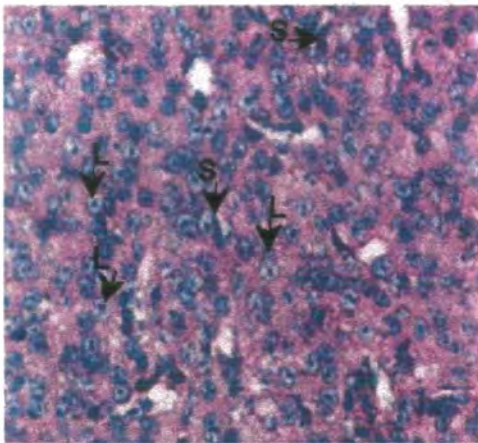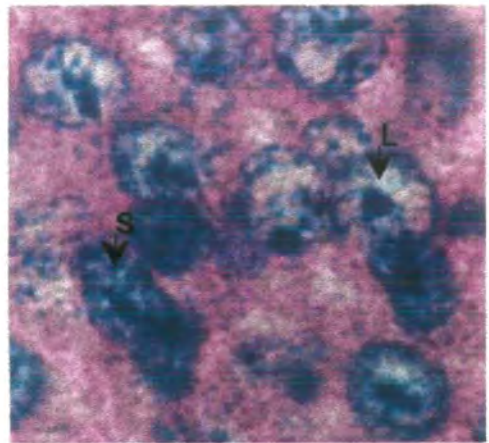
Figure 13: Photomicrograph of the rat ovary at 120 days of age showing corpus luteum. Control ×330 **(A)** same at higher magnification **(B)** showing large (L) and small (S) luteal cells. Postnatal thyroxine × 832 **(C)** postnatal PTU ×330 **(D)** same at higher magnification × 832 **(E)** treatment group with greater number of large luteal cells (L).

TABLE 14: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT DURING PRE- AND POSTNATAL PERIOD ON GRAAFIAN FOLLICLES, OOCYTE AND THICKNESS OF PERIPHERAL GRANULOSA LAYER OF FEMALE RAT AT 120 DAYS OF AGE.

| Groups n=10	Diameter of Graffian Follicles (µm)	Diameter of Oocyte (µm)	Thickness of Peripheral Granulosa Layer (µm)
Control	414.80 ± 21.61	49.38 ± 1.99	90.00 ± 4.70
Prenatal Treatment			
PTU	585.40 ± 24.03[a***]	52.71 ± 2.55	109.20 ± 4.99
Malathion	393.20 ± 20.53	56.14 ± 2.42	81.82 ± 5.85
Thyroxine	396.70 ± 22.63	53.80 ± 2.28	85.33 ± 6.16
Postnatal Treatment			
PTU	396.00 ± 26.00[b***]	46.75 ± 2.38	77.00 ± 4.48[b***]
Malathion	491.00 ± 21.96	52.00 ± 3.38	92.67 ± 3.00
Thyroxine	488.00 ± 17.52	56.00 ± 1.55	82.00 ± 6.96

Values (Mean ± SEM)
a= treated groups vs control
b= prenatal vs postnatal PTU treatment groups
P<0.001***
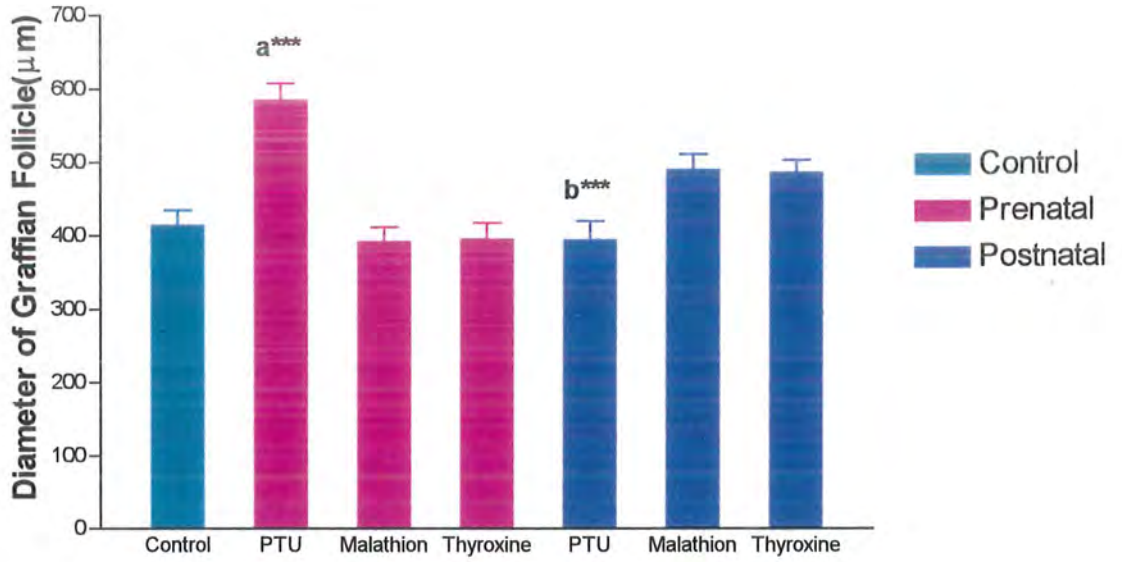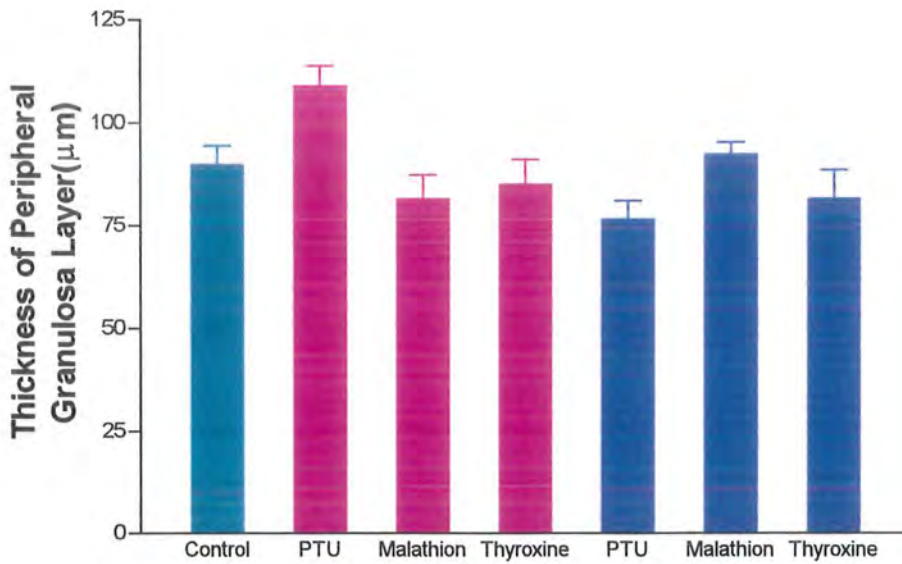
Figure 14: Diameter of graffian follicles **(A)** of control and treated rats at 120 day of age. Data are expressed as Mean ± SEM. Control vs pre- and postnatal treatment groups **(a)**.Prenatal PTU treatment show a significant *** P<0.001 increase compared to control **(a)** and postnatal PTU treatment group **(b)**. Thickness of peripheral granulosa layer **(B)** shows non significant (P>0.05) difference between control, pre- and postnatal treatment groups.

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THICKNESS OF PERIPHERAL GRANULOSA LAYER:

Total mean thickness of peripheral granulosa layer of control, pre- and postnatal PTU, malathion and thyroxine treated ovaries are shown in Table 14; Figure 14B Thickness of peripheral granulosa layer of prenatal PTU treatment group (109.20± 4.99 µm) show a slight but non significant (P>0.05) increase as compared to control (90.00 ± 4.70 µm). In contrast, thickness of granulosa layer of postnatal PTU treatment group (77.00 ± 4.49 µm) exhibit highly significant (P<0.001) reduction as compared to prenatal PTU treatment group. Thickness of granulosa layer of prenatal malathion treatment group (81.82 ± 5.85 µm) is also reduced as compared to control, but this difference is not appreciable (P>0.05).

All other pre- and postnatal treatment groups show no significant (P>0.05) difference in thickness of peripheral granulosa layer in comparison to control.

DIAMETER OF PRIMARY FOLLICLES AND OOCYTE:

Mean diameter of primary follicles of control, pre- and postnatal treatment group are shown in Table 15; Figure 15. Diameter of primary follicles ranged from 50-100 µm. There is no significant (P>0.05) difference in mean diameter of primary follicles of control, pre- and postnatal PTU, malathion and thyroxine treatment groups. Although postnatal PTU treatment group (92.50 ± 2.55 µm) show slight increase, and postnatal malathion treatment group (67.31 ± 2.55 µm) exhibit slight decrease in diameter of primary follicles as compared to diameter of control

TABLE 15: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT
DURING PRE- AND POSTNATAL PERIOD ON DIAMETER
OF PRIMARY FOLLICLES AND OOCYTE OF FEMALE RAT
AT 120 DAYS OF AGE.

Groups n=10	Diameter of Primary Follicle (μm)	Diameter of Oocyte (μm)
Control	82.32 ± 4.31	30.00 ± 2.63
Prenatal Treatment		
PTU	88.89 ± 4.02	36.67 ± 2.92
Malathion	83.25 ± 5.65	31.00 ± 2.89
Thyroxine	81.46 ± 3.74	31.46 ± 2.43
Postnatal Treatment		
PTU	92.50 ± 2.56	35.00 ± 2.38
Malathion	67.31 ± 4.61	28.08 ± 2.61
Thyroxine	87.73 ± 2.83	30.68 ± 1.91

Values (Mean ± SEM)
There is no significant difference($P > 0.05$) in mean diameter of primary follicles
and oocyte of control ,pre- and postnatal treatment groups.

Figure 15: Diameter of primary follicles of control and treated female rats at 120 day of age. Data are expressed as Mean ± SEM. There is non significant (P>0.05) difference between control, pre- and postnatal treatment groups.

(82.32 ± 4.30 µm). But this variation in diameter of primary follicles is not appreciable (P>0.05).

There is no significant (P>0.05) difference in oocyte of primary follicles of control, pre- and postnatal PTU, malathion and thyroxine treatment groups (Table 15).

DIAMETER OF DEVELOPING FOLLICLES AND OOCYTE:

Developing follicles are categorized into different groups with respect to the diameter. These groups ranging from 101-150, 151-200, 201-250, 251-300 and 301-350 µm. Mean diameter of these different groups of developing follicles shows small but non significant (P>0.05) variation in control, pre- and postnatal PTU, malathion and thyroxine treatment groups (Table 16; Figure 16).

Mean diameter of oocyte of different ranges of developing follicles show no significant (P>0.05) difference in control, pre- and postnatal PTU, malathion and thyroxine treatment group (Table 17).

NUMBER OF OVARIAN FOLLICLES:

GRAFFIAN FOLLICLES:

Number of graafian follicles in control, pre- and postnatal PTU, malathion and thyroxine treated ovaries are shown in Table 18. There is no appreciable difference (P>0.05) in mean number of graafian follicles of control, pre- and

TABLE 16: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT DURING PRE- AND POSTNATAL PERIOD ON DIAMETER OF DEVELOPING FOLLICLES OF FEMALE RAT AT 120 DAYS OF AGE.

Groups n=10	Different Ranges of Developing Follicles				
	101-150 µm	151-200 µm	201-250 µm	251-300 µm	301-350 µm
Control	129.20 ± 3.08	185.00 ± 10.36	210.60 ± 3.44	275.00 ± 0.00	———
Prenatal Treatment					
PTU	120.50 ± 5.28	177.10 ± 3.76	233.80 ± 7.40	267.50 ± 7.43	336.70 ± 8.46
Malathion	140.00 ± 2.66	174.50 ± 6.63	———	257.50 ± 0.00	———
Thyroxine	121.40 ± 5.00	169.00 ± 4.37	221.80 ± 5.77	275.00 ± 20.00	———
Postnatal Treatment					
PTU	120.30 ± 5.83	175.00 ± 6.85	213.80 ± 1.25	———	340.00 ± 0.00
Malathion	122.90 ± 6.31	185.80 ± 12.94	215.50 ± 3.91	260.60 ± 3.13	320.00 ± 0.00
Thyroxine	116.10 ± 4.85	188.80 ± 2.17	213.80 ± 6.25	262.50 ± 0.00	312.50 ± 0.00

Values (Mean ± SEM)

There is no significant difference(P>0.05) in mean diameter of different ranges of developing follicles of control, pre- and postnatal treatment groups.
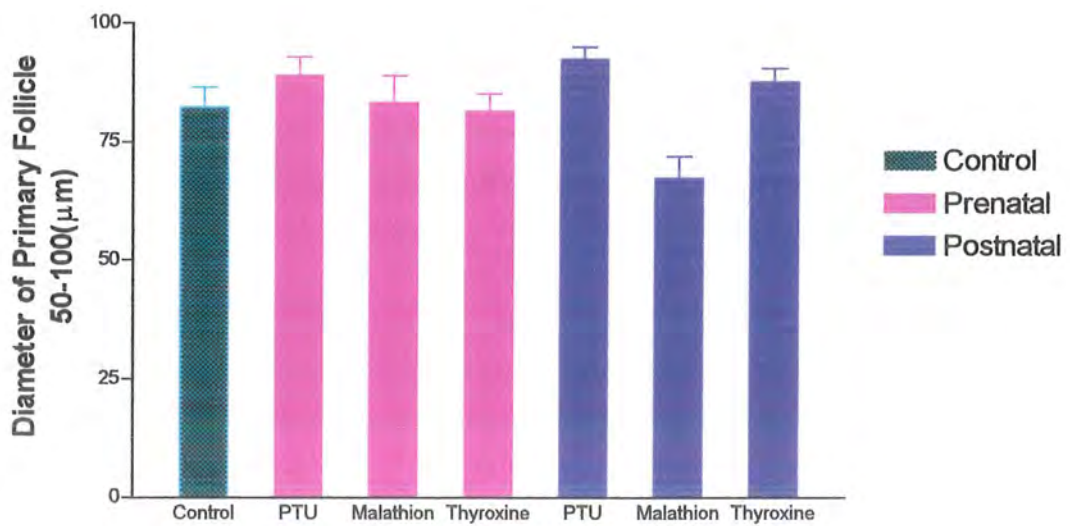
——— = absence of follicles.
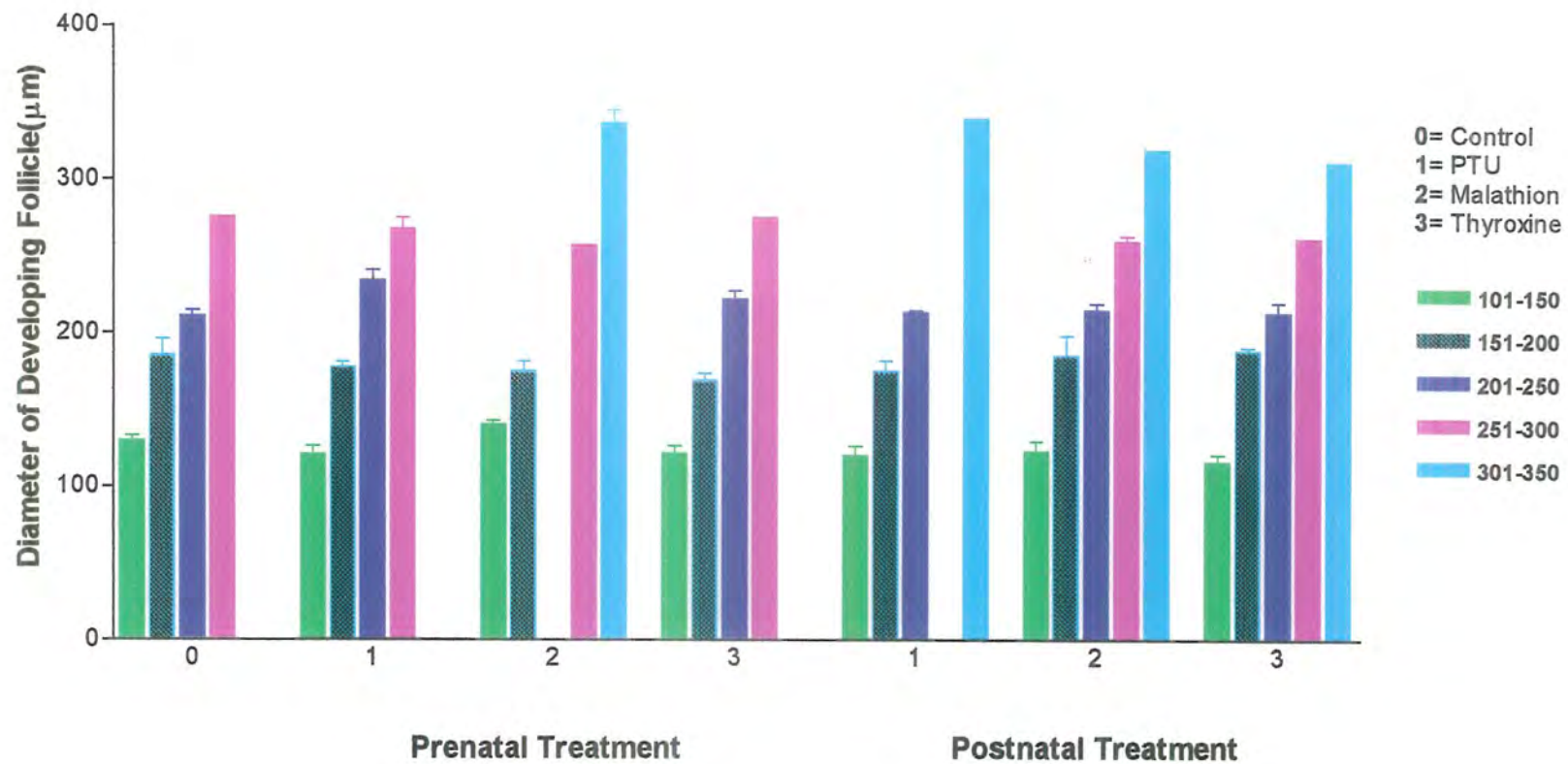
Figure 16: Diameter of developing follicles of control and treated female rats at 120 day of age. Data are expressed as Mean ± SEM. There is non significant (P>0.05) difference between control, pre- and postnatal treament groups.

TABLE 17: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT DURING PRE- AND POSTNATAL PERIOD ON DIAMETER OF OOCYTE OF DEVELOPING FOLLICLES OF FEMALE RAT AT 120 DAYS OF AGE.

Groups n=10	Diameter of Oocyte of Developing Follicles				
	101-150 μm	151-200 μm	201-250 μm	251-300 μm	301-350 μm
Control	42.88 ± 1.83	48.13 ± 3.44	51.25 ± 2.60	57.50 ± 0.00	————
Prenatal Treatment					
PTU	44.10 ± 1.90	52.50 ± 3.18	61.88 ± 3.73	56.25 ± 8.57	61.67 ± 4.64
Malathion	44.17 ± 5.19	47.50 ± 3.26	————	55.00 ± 0.00	————
Thyroxine	39.39 ± 1.89	49.50 ± 3.30	51.79 ± 2.48	50.00 ± 7.50	————
Postnatal Treatment					
PTU	43.06 ± 3.28	53.13 ± 7.39	57.50 ± 0.00	————	45.00 ± 0.00
Malathion	36.25 ± 2.87	36.67 ± 5.47	45.00 ± 1.77	53.75 ± 5.05	62.50 ± 0.00
Thyroxine	43.21 ± 5.02	49.38 ± 3.59	52.50 ± 2.50	35.00 ± 0.00	62.50 ± 0.00

Values (Mean ± SEM)
There is no significant difference (P>0.05) in mean diameter of oocyte in different ranges of developing follicles of control ,pre- and postnatal . treatment groups. ————= absence of follicles and their oocytes.

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postnatal PTU, malathion and thyroxine treatment groups. Although the number of follicles of postnatal PTU (2.5±0.64) and thyroxine (2.5±0.86) treatment groups is slightly decreased as compared to control (5.00±0.58), but the variation is not significant (P>0.05).

PRIMARY FOLLICLES:

Mean number of primary follicles present in the ovaries of the rats in pre- and postnatal PTU, malathion and thyroxine treatment groups although vary from control, but this difference is not significant (P>0.05) as shown in Table18.

DEVELOPING FOLLICLES:

Number of different ranges of developing follicles in control, pre-and postnatal PTU, malathion and thyroxine treatment groups are shown in Table 18. Pre-and postnatal PTU, malathion and thyroxine treatment groups have no significant (P>0.05) difference in mean number of developing follicles in the range of 101-150μm as compared to control. Prenatal and postnatal malahtion (1.50 ± 0.86, 1.50±0.64) and postnatal PTU (1.50 ± 0.50) show a slight but non significant (P>0.05) decrease in number of developing follicles (101-150μm) as compared to control (3.25 ± 0.94).

TABLE 18: EFFECT OF PTU, MALATHION AND THYROXINE TREATMENT DURING PRE- AND POSYNATAL PERIOD ON THE NUMBER OF OVARIAN FOLLICLES OF FEMALE RAT OVARY AT 120 DAYS OF AGE.

Groups n=10	Number of Graafian Follicles (351-600µm)	Number of Primary Follicles (50-100µm)	Number of Developing Follicles				
			101-150 µm	151-200 µm	201-250 µm	250-300 µm	301-350 µm
Control	5.00 ± 0.58	2.75 ± 0.25	3.25 ± 0.95	1.00 ± 0.58	1.00 ± 0.41	0.25 ± 0.25	———
Prenatal Treatment							
PTU	3.00 ± 0.41	2.25 ± 0.75	2.50 ± 0.87	1.75 ± 0.48	1.00 ± 0.41	1.00 ± 0.41	———
Malathion	2.75 ± 0.63	2.50 ± 1.19	1.50 ± 0.87	1.25 ± 0.95	———	0.25 ± 0.25	0.75 ± 0.75
Thyroxine	3.75 ± 0.48	3.00 ± 0.82	2.25 ± 0.48	1.50 ± 0.87	1.50 ± 0.87	0.50 ± 0.29	———
Postnatal Treatment							
PTU	2.50 ± 0.65	2.75 ± 0.75	1.50 ± 0.50	1.25 ± 0.25	0.50 ± 0.29	0.25 ± 0.25	0.25 ± 0.25
Malathion	3.75 ± 1.25	3.25 ± 0.48	1.50 ± 0.65	0.75 ± 0.25	1.25 ± 0.75	1.00 ± 0.71	0.25 ± 0.25
Thyroxine	2.50 ± 0.87	4.50 ± 0.50	2.75 ± 0.63	2.00 ± 0.91	0.75 ± 0.48	0.25 ± 0.25	0.25 ± 0.25

Values (Mean ± SEM)

There is no significant difference (P>0.05) in mean number of graafian, primary and developing follicles of control, pre- and postnatal treatment group.

——— = absence of follicles.

Developing follicles ranged from (301-350μm) are not found in control, prenatal PTU and thyroxine treatment groups. Other treated groups in which these follicles are present show no significant (P>0.05) difference to one another.

Mean number of all other different ranges of developing follicles does not have any significant (P>0.05) difference in pre-and postnatal PTU, malathion and thyroxine treatment groups as compared to control.

SERUM ESTRADIOL:

Serum estradiol concentration of control, pre-and postnatal PTU, malathion and thyroxine treatment group is shown in Figure 17. All the pre- and postnatal treatment groups show a slight reduction in serum estradiol levels as compared to control although the reduction is statistically non significant (P>0.05).
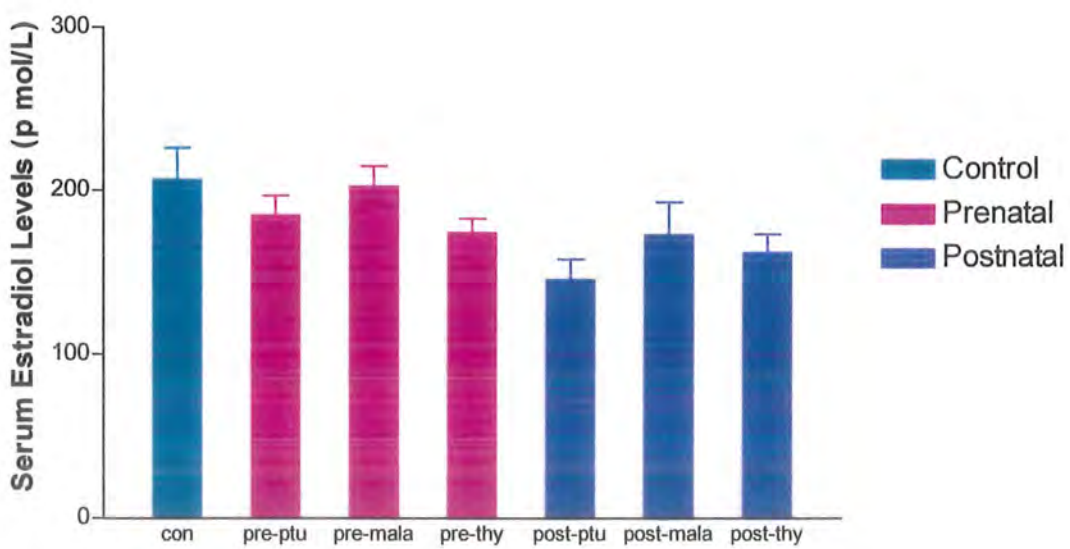
Figure 17: Serum estradiol concentration of control and treated rats at 120 day of age. Data are expressed as Mean ± SEM. There is no significant (P>0.05) difference in control, pre- and postnatal PTU, malathion and thyroxine treatment groups.

DISCUSSION

In present study hypothyroidism is induced by giving 0.1% PTU in drinking water of mothers, as also used by different investigators (Cooke et al., 1992; Cooke et al., 1993; Mendis-Handagama and Sharma, 1994; Hardy et al., 1996; Simorargkir et al., 1997).It has been observed in present study that PTU treatment to mothers from conception to parturition reduces their pregnancies and litter size. These findings are in accordance with the results reported by Varma et al (1978) that maternal hypothyroidism resulted in fewer pregnancies and small litter size. The mothers in present study receiving PTU from conception to parturition have reduction in feed and water consumption during the treatment period. It has been observed by Cooke et al (1993) that mothers receiving 0.1% PTU from parturition to 25 day postpartum showed reduction in uptake water during the treatment period. The result of this study indicates an interesting phenomenon that mothers receiving 0.1% PTU from conception to parturition have reduction not only in water uptake but also in feed consumption during the treatment period. These treated mothers gained little weight during the treatment due to the small intake of feed and water during the gestation period as compared to control mothers. As a consequence prenatal PTU treated pups have small but non significant reduction in body weight only at the time of birth as compared to control.

Present study revealed that with in one week after the withdrawal of prenatal PTU treatment, mothers have marked increase in their feed consumption, water uptake and body weight. As the diet and health of mothers improved gradually, there was gradual increase in body weight of their pups. At the end of present experiment,

body weight of these treated pups are comparable to those of control. Therefore, it is concluded that PTU treatment during the prenatal period of life has a reversible effect on feed consumption, water uptake and body weight during the postnatal period of life.

On the other hand, postnatal PTU treatment shows different results. Marked decrease in feed consumption, water uptake and body weight of mothers receiving PTU from parturition to 25 days postpartum is observed during the treatment period. Pups of these mothers start to loose their body weight along with the initiation of the treatment. These treated mothers refuse to take sufficient amount of water even after the addition of Rooh-Afza to minimize the bitterness of PTU. Cooke et al (1993) also observed decreased water uptake of mothers receiving 0.1% PTU from parturition to 25 days postpartum even after the addition of some agent to give good taste to water.

Low survival rate (30%) of postnatal PTU treated pups is examined in the present study. It is possible that reduction in feed and water consumption of the mothers receiving PTU from parturition to 25 days postpartum leads to low amount of milk production (containing sufficient amount of PTU), which does not fulfil the nutritional requirement of their pups. Transfer of PTU through milk was reported by Kawada et al (1988) and Cooke et al (1993). Many of the pups die (70%) due to insufficient lactation and remaining alive (30%) have retarded growth and physical development. Different scientists studied the effect of PTU during postnatal period of life and reported, that the postnatal PTU treated pups have

retarded growth and physical development, delay in eye opening and teething, slow in responding to general environment and also have depressed body weight as compared to control (Meisami, 1984; Tamasy et al., 1984; Kawada et al., 1988; Akaike et al., 1991; Madeira et al., 1991; Cooke et al., 1992; Madeira et al., 1992; Madeira and Paula-Barbosa, 1993; Akaike and Kato, 1997). Result of present study are in agreement with the findings of all the above mentioned scientists, but interestingly it is noticeable in this study that postnatal PTU treated pups also have delay in fur development as compared to control.

In current study control pups are active in response and behaviour at the time of weaning (28-30 days). While postnatal PTU treated pups are less active in response and behaviour at the time of weaning which is initiated between 35-37 days postpartum. This delay in weaning process by 0.001% and 0.1% PTU was previously reported by Blake et al (1985). One possible reason for this delay is that the pups start to take their feed themselves at a later stage. As they have depressed growth and physical development as compared to control.

It is concluded from the present study that after the withdrawal of treatment, postnatal PTU treated pups although show gradual increase in growth, physical development and body weight but they are still smaller than control at 120 day of age. Similar decrease in body weight of postnatal PTU treated rats was observed by Blake et al (1985) with 0.01% and 0.001% PTU at 28 day of age and Dijkstra et al (1996) with 0.1% PTU at 40 day of age.

It is documented by the present study that pre- and postnatal malathion treatment have no profound effect on body weight of treated pups. Akhtar et al (1996) have studied that administration of 0.06mg malathion to adult rats for 21 days does not effect their body weight. Effectiveness of malathion was studied in mice by Mufti and Asmatullah (1997). They documented that administration of high doses of malathion (233.3µg/g BW) to mothers caused foetal mortality, sometime foetal resorption and decrease in foetal body weight at 15th day of gestation. Pluth et al (1996) reported growth retardation in rats fed with high doses of malathion(240mg/Kg/day).

Normal survival, growth and development of pre- and postnatal malathion treated pups have been observed in the present study. Similar results have been obtained by Clemens et al (1990) working with another organophosphate, Metasystox-R(MSR) (0.5, 1.5, 4.5mg/Kg)given from6-15 day postpartum. Present study indicates that dose of 0.06mg malathion is too small to cause any alteration in feed consumption, water uptake and body weight of pups. High doses of malathion used over long period of time have embryo toxic and feto toxic effect or cause profound developmental abnormality such as polydactyle in mice (Mufti and Asmatullah, 1997).

It was previously reported that thyroid hormones regulate the body metabolism, physical development and growth (Guyton, 1996). It was reported by Gill (1991) that hyperthyroidism increase the metabolic rate and decreased the body weight.

It was reported by Besa and Pascual Leone (1984) that high doses of thyroxine administered to rats during their first week of life leads to a decrease in body weight. Current results corresponds to this finding, as postnatal thyroxine treated pups have significant (P<0.01) decrease in body weight. Decrease in body weight of male rats was found by Van Haaster et al (1993) by inducing hyperthyroidism with subcutaneous injection of 100µg triiodothyronine/Kg BW from 1-16 day postpartum. Interestingly it is examined in the present study that prenatal thyroxine treatment (0.25µg) also causes reduction in body weight as compared to control. It is concluded that exogenous supply of thyroxine irrespective of pre- and postnatal treatment, is responsible for reduction in body weights of female pups at 120 day of age.

It has also been noted in present study that prenatal PTU treatment caused a slight but non significant (P>0.05) increase in ovarian weight as compared to control. The consequences of hypothyroidism on the ovaries of rats have recently been studied by Dijkstra et al (1996). They induced hypothyroidism in prepubertal rats by giving 0.1% PTU in drinking water of dams and pups from 0-40 day postpartum. Lower ovarian and body weight of hypothyroid rats was observed at 21 and 40 day of age. Decrease in ovarian and body weight of postnatal PTU treated hypothyroid rats has also been observed in present study at 120 day of age. The result of present and previous (Dijkstra et al., 1996) study indicate that postnatal PTU treatment from birth onward resulted in lowered body weight and gonadal weight not only in prepubertal rats (40 day of age) but also in pubertal

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rats (120 day of age). Therefore it is concluded that gonadal weight of an animal has a correlation to its body weight.

Regarding the number of ovarian follicles in 40 day old hypothyroid rats, Dijkstra et al (1996) found that their ovaries contain 13.1% more secondary follicles with only two or three layers of granulosa cells and less antral follicles. Chan and Ng (1995) studied the effect of hypothyroidism induced by PTU (50µg/g BW) on reproductive system of female mice at 14, 21 and 28 day postpartum. According to the result of their study, postnatal PTU treatment reduces the number of primordial, multilaminar and graafian follicles at 14, 21 and 28 day of age. Present study revealed no change in number of primordial follicles of postnatal PTU treated rats at 120 days of age compared to control. Whereas, small but statistically non significant (P>0.05) reduction has been found in number of developing follicles and graafian follicles at 120 days of age compared to control. Mattheij et al (1995) also reported that hypothyroidism reduces the number of follicles which are able to ovulate.

Present study revealed no difference in structure of primary, developing and graafian follicles compared to control. However, polyovular follicles have been observed in the ovaries of postnatal PTU treatment group. Polyovular follicles in rats have already been observed by Peters (1978). The development of polyovular follicle is not clear. It might be formed during the process of follicular organization. It could be possible that they represent some phenomenon of

follicular atresia. Or deficiency of estrogen is responsible for their development (Peters, 1978).

No difference was found in mean diameter of various classes of secondary follicles and their oocyte of postnatal PTU treated rats at 40 day of age compared to control(Dijkstra et al., 1996). Results of present study correlate to this finding in the sense, that postnatal PTU treatment has no effect on diameter of developing follicles and their oocyte at 120 day of age compared to control. No effect have also been found on diameter of primary follicles.

It was reported by Greenwald (1978) that in normal rats, follicles having diameter larger than 400μm have an antral cavity. In current study the diameter of graafian follicles in control ovary is found to be 414.80μm which correspond to this finding. Dijkstra et al (1996) noticed, increased diameter of advanced antral follicles in postnatal PTU treated rats at 40 day of age compared to control. It has been found by the present study that prenatal PTU treatment caused a significant increase (P<0.001) in diameter of graafian follicle while, postnatal PTU treatment caused a slight but non significant (P>0.05) reduction in diameter compared to control.

Present study revealed that graafian follicle of prenatal PTU treatment exhibit a slight but non significant (P>0.05) increase in thickness of their peripheral layers of granulosa cells compared to control. Whereas, postnatal PTU treatment caused small but non significant (P>0.05) decrease in thickness of peripheral

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granulosa layer as compared to control. From these result it can be suggested that thickness of peripheral granulosa layer is one possible cause for increasing or decreasing the diameter of graafian follicles. Size of antral cavity, antral fluid and oocyte also contribute to the diameter of graafian follicles (Johnson and Everitt, 1995).

Current study revealed increase in body weight, ovarian weight and diameter of graafian follicle of prenatal PTU treatment compared to control. While postnatal PTU treatment show reduction in body weight, ovarian weight and diameter of graafian follicles compared to control. These result corresponds to the finding of Espey (1978) that in a given species, the size of mature graafian follicle is proportional to the body weight of female.

Roelofs and Kramer (1977) documented that first ovulation occurs only in rats with a body weight over 90gm. It is well established fact that formation of corpus luteum takes place after the ovulation in mammals (Niswender and Nett 1994). In this study number of corpora lutea are found both in pre- and postnatal PTU treated rats at 120 day of age. These pre- and postnatal PTU treated rats have body weights 155 ± 21.57 and $109 \pm 17.97g$ respectively which is greater than 90 gm.Corpora lutea of normal rats contain large and small luteal cells in equal proportion (Niswender and Nett, 1994) The corpora lutea of postnatal PTU treated rats in the present study also contain more population of large luteal cells as compared to control. Presence of luteal cells is indicative of steroidogenic activity of corpora lutea (Niswender and Nett 1994).

There is very little information regarding the effect of hypothyroidism induced by malathion on ovarian function and structure in female rats. Present study revealed no effect of malathion during the pre- and postnatal period of life on ovarian morphology, histology and morphometery. Ovarian weight, size, diameter and number of primary, developing and graafian follicles and corpora lutea of pre- and postnatal malathion treated groups are more or less similar to that of control. Ozmen and Akay (1993) found no histopathological changes in ovaries of rats after giving two dosage levels of malathion (10 and 100mg/Kg/day) to adult rats for 15 weeks. Ahmad et al (1993) studied the effect of another organophosphate on ovarian function and structure of rats. He found that administration of chlorpyrifos (7 and 14mg/Kg/day) to neonates rats from 8-23 day postpartum decreases the weight of female reproductive organs. It is concluded that dosage level of malathion (0.06mg) used in this study is too small to exert any toxic effect on ovarian function and structure.

Scanty information is available concerning the effect of pre- and postnatal thyroxine treatment on ovarian function and structure. In present study hyperthyroidism is induced during postnatal period of life by giving daily subcutaneous injection of 0.3µg thyroxine/g B.W to female pups from birth to 25 day postpartum. Subcutaneous injection of thyroxine to induce hyperthyroidism was also used by different investigators (Lakshmanan et al., 1986; Lakshmanan and Landel, 1986; Paternostro and Meisami, 1991; Dipple et al., 1993). At the end of experiment (120 day of age) decrease in ovarian weight is observed. These

results are in agreement to the findings of Besa and Pascual-Leone (1984) that the administration of thyroxine during the first week of life results in decreased gonadal weight compared to control. These results also coincide with the work of Fitko and Szlezyngier (1994) who noticed diminished ovarian size in hyperthyroid rats in comparison to control. Pre- and postnatal thyroxine treatment exert no effect on number and diameter of primary, developing and graafian follicles compared to control. Polyovular follicles also observed in postnatal thyroxine treatment group. Regarding the corpora lutea, in postnatal thyroxine treatment greater number of large luteal cells have been found compared to control.

In present study concentration of serum estradiol shows no difference among control, pre- and postnatal PTU, malathion and thyroxine treatment groups. Although postnatal PTU and thyroxine treatment group have a small decrease in serum estradiol concentration as compared to control, but this decrease is statistically non significant (P>0.05). In control, pre- and postnatal PTU, malathion and thyroxine treated groups, there was no difference in the number of ovarian follicles and thickness of peripheral layer of granulosa cells. Finally it is possible that PTU, malathion and thyroxine do not exert any effect on FSH and LH receptors present on the granulosa cells to alter estrogen production. The granulosa cells are involved in steroidogenic activity (Johnson and Everitt, 1995).Hence, there is no significant (P>0.05) difference in serum estradiol concentration of control, pre- and postnatal PTU, malathion and thyroxine treatment groups.

In summary it is concluded from the present study that successful treatment of 0.1% PTU to immature female rats from 0-25 day postpartum effect the growth, physical development caused a decrease in body weight and ovarian weight at 120 day of age. Treatment of immature rats with $0.3\mu g$ thyroxine/g BW from 0-25 day postpartum also decrease the body and ovarian weight. This indicate the importance of thyroid hormone during the early postnatal period of life regarding the growth, physical development and ovarian weight. While pre- and postnatal malathion and prenatal PTU and thyroxine have no profound effect on ovarian morphology, histology and morphometery. All the treatment during the pre- and postnatal period of life do not alter the concentration of estradiol at 120 day of age.

REFERENCES

Ahmad MM, Ahmad MM and Sarvat S 1993 Effects of endosulfan and Chlorpyrifos on the reproductive organs and sex hormones of neonatal rats. Pakistan J Zool 25: 11-14

Akaike M and Kato N 1997 Abnormal behaviour, spatial learning impairment and neuropeptides caused by temporary neonatal hypothyroidism. Recent Res Dev Neuroendo 39-48

Akaike M, Kato N, Ohno H and Kobayashi T 1990 Hyperactivity and spatial maze learning impairment of adult rats with temporary neonatal hypothyroidism. Neurotoxicology and Teratology 13: 317-322

Akhtar N, Kayani SA, Ahmad MM and Shabab M 1996 Insecticide–induced changes in secretory activity of the thyroid gland in rats. J Appl Toxicol 16: 397-400

Balasubramanian K, Vijayan AP, Aanathanarayanan PH and Balasubrama nian A 1987 Effect of malathion on the testis of male albino rats. Med Sci Res 15: 229-230

Balasubramanian K, Vijayan AP, Ananthanarayanan PH and Balasubramanian A 1986 Effect of malathion on the thyroid function of male albino rats. IRCS Med Sci 14: 1139-1149

Beard MD and Mackay Sim A 1987 Loss of sense of smell in adult hypothyroid mice. Dev Brain Res 36: 181-189

Besa ME and Pascual-Leone AM 1984 Effect of neonatal hypothyroidism upon the regulation of TSH secretion in rats. Acta Endocrinologica 105: 31-39

Blake, Helen H and Henning SJ 1985 Effect of propylthiouracil dose on serum thyroxine growth and weaning in young rats. AM J Physiol 248 (5 part 2): R524-R530

Chan WY and Ng TB 1995 Effect hypothyroidism induced by propylthiouracil and thiourea on male and female reproductive systems of neonatal mice. J Exp Zool 273: 160-169

Clemens GR, Hartnagle RE, Bare JJ and Thyssen JH 1990 Teratological, neurochemical, and postnatal neurobehavioral assessment of Metasystox-R, and organophosphate pesticide. Fundam Appl Toxicol 14: 131-143

Clos J, Crepel F, Legrand C, Rabie A and Vigouroux E 1974 Thyroid physiology during the postnatal period in the rat: A study of the development of thyroid function and of the morphogenetic effects of thyroxine with special reference to cerebellar maturation. General and Comparative Endocrinology 23: 178-192

Cooke PS, Kirby JD and Procelli J 1993 Increased testis growth and sperm production in adult rats following transient neonatal goitrogen treatment: Optimization of the propylthiouracil dose and effects of methimazole. J Reprod Fertil 97: 493-499

Cooke PS, Porcelli J and Hess RA 1992 Induction of increased testis growth and sperm production in adult rats by neonatal administration of goitrogen propylthiouracil (PTU): The critical period. Biology of Reproduct- ion 46: 146-154

Coulombo P, Ruol J and Dussault JH 1980 Effects of neonatal hypo- and hyperthyroidism on pituitary growth hormone content in the rat. Endocrinology 107: 2027-2033

Deborah WA, Millar LK, Koonings PP, Montoro MN and Mestman JH 1994 A comparison of propylthiouracil versus methimazole in the treatment of hypothyroidism in pregnancy. Am J Obste Gynecol 170: 90-95

Dijkstra G, De Rooij DG, De Jong FH and Ven Den Hurk R 1996 Effect of hypothyroidism on ovarian follicular development, granulosa cell proliferation and peripheral hormone levels in the prepubertal rat. European Journal of Endocrinology 134: 649-654

Dipple KM, Qulali M, Ross RA and Crabb DW 1993 Effects of thyroxine on the expression of alcohol dehydrogenase in rat liver and kidney. Hepatology 17: 701-706

Drury RAB and Wallington EA 1980 Carleton's histological techniques, Oxford University Press, Great Britain, pp 57-150

Espey LL 1978 Ovulation. In: Jones RE (ed) The vertebrate ovary compararative biology and evolution, Plenum Press, New York, pp 504-505.

Fitko R and Szlezyngier B 1994 Role thyroid hormone in controlling the concentration of luteinizing hormone/ human chorionic gonadotropin receptor in rat ovaries. Eur J Endocrinol 130: 378-380

Gentile F, Lauro R and Salvatore G 1995 Biosynthesis and secretions of thyroid hormones. In: DeGroot LJ (eds) Endocrinology, W D Sunders, Philadelphia, pp 517-542

Gill GN 1991 The thyroid gland. In: West JB (ed) Physiological basis of medical practice, Williams and Wilkins, London, pp 811-818

Greenwald GS 1978 Follicular activity in the mammalian ovary. In: Jones RE (ed) The vertebrate ovary comparative biology and evolution, Plenum Press, New York, pp 664-666.

Guedes RCA and Pereira-Da-Silva MS 1993 Effect of pre- and postnatal propylthiouracil administration on the prolongation of cortical spreading depression of adult rats. Brazilian Journal of Medical and Biological Research 26: 1123-1128

Guyton C 1996 The thyroid metabolic hormones. In: Text book of medical physiology, W B Saunders, Philadelphia, pp 831-839

Hardy MP, Sharma RS, Arambepola NK, Sttos CM, Russell LD, Bunick D, Hess RA and Cooke PS 1996 Increased proliferation of Leydig cells induced by neonatal hypothyroidism in the rat. J Androl 17: 231-238

Husain K, Adil Mirza M and Matin MA 1989 Influence of chloramphenicol on certain acute toxic effects induced by malathion in rats. Biol Mem 15: 143-146

Johnson MH and Everitt BJ 1995 Ovarian function. In: Essential reproduction Blackwell Science Ltd, London, pp 61-77

Johnson MH and Everitt BJ 1995 The fetus and its preparations for birth. In: Essential reproduction, Blackwell Science Ltd, London, pp 190-207

Kalland GA, Vera A, Peterson M and Swerdloff RS 1978 Reproductive hormonal axis of the male rat in experimental hypothyroidism. Endocrinology 102: 476-484

Kawada, Jun Mino H, Nishida M and Yoshimura Y 1988 An appropriate model for congenital hypothyroidism in rat induced by neonatal treatment by propylthiouracil and surgical thyroidectomy: Studies on learning ability and biochemical parameters. Neuroendocrinology 47: 424-430

Kirby JD, Jetton AE, Cooke PS, Hess RA, Bunik D, Ackland JF, Turek FW and Schwartz NB 1992 Developmental hormonal profiles accompanying the neonatal hypothyroidism-induced increase in adult testicular size and sperm production in the rat. Endocrinology 131: 559-565

Kirby JD, Mankar MV, Hardesty D and Kreider DL 1996 Effects of transient prepubertal 6-n- propyl-2-thiouracil treatment on testis development and function in the domestic fowl. Biology of Reproduction 55: 910-916

Lakshmanan J and Landel CP 1986 Neonatal hyperthyroidism impairs epinephrine-provoked secretion of nerve growth factor and epidermal growth factor in mouse saliva. Pediatr Res 20: 587-592

Lakshmanan J, Perheentupa J, Alm J and Fisher DA 1986 Neonatal hyperththyroidism in mice has different effects on epidermal growth factor levels in submandibular gland, urine, and blood. Pediatr Res 20: 628-631

Larsen PR and Ingbar SH 1992 The thyroid gland. In: Wilson JD and Foster DW (eds) Williams text book of Endocrinology, W B Saunders Company, Philadelphia, pp 357-487

Lechner, Waldron DM and Abdel-Rahman MS 1984 A teratology study of carbaryl and malathion mixture in rats. J Toxicol Environ Health 14: 267-278

Mackay-Sim A and Beard MD 1987 hypothyroidism disrupts neural development in the olfactory epithelium of adult mice. Dev Brain Res 36: 190-198

Madeira MD, Cadet–Leite A, Andrade JP and Paula-Barbosa MM 1991 Effect of hypothyroidism upon the granular layer of the dentate gyrus in male and female adult rats: A morphometeric study. The Journal of Comp- arative Neurology 314: 171-186

Madeira MD, Paula-Barbosa MM 1993 Reorganization of mossy fiber synapses in male and female hypothyroid rats: A sterological study. The Journal of Comparative Neurology 337: 334-352

Niswender GD and Nett TM 1994 The corpus luteum and its control in infraprimate species. In: Knobil E (eds) The physiology of reproduction, Raven Press, New York, pp 781-816

Ortega E, Rodriguez E, Ruiz E and Osorio C 1990 Activity of the hypothalamo-pituitary ovarian axis in hypothyroid rats with or without triiodothyronine replacment. Life Sci 46: 391-395

Ozmen G and Akay MT 1993 The effects of malathion on some hormone levels and tissue secreting these hormones in rats. Vet Hum Toxicol 35: 22-24

Paternostro MA and Meisami E 1991 Lack of thyroid hormones but not their excess affects the maturation of olfactory receptors neurons: A quantitative morphologic study in the postnatal rats. Int J Dev Neurosci 9: 439-452

Peters H 1978 Folliculogenesis in mammals. In: Jones RE (ed) The vertebrate ovary comparative biology and evolution, Plenum Press, New York, pp 132-133.

Pluth J, Nicklas J, O'Neill P and Albertini R 1996 Increased frequency of specific genomic deletions resulting from malathion exposure. Cancer Research 65: 2393-2399