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Effect of Hypothyroidism Induced by Propylthiouracil on Ovarian Function and Structure in Offspring From Treated Mothers (Rats)

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ABSTRACT The aim of the present study was to investigate the effects of hypothyroidism induced during the pre- and postnatal periods of life on ovarian function and structure in offspring (pups) 120 days of age. Three groups were used. In the prenatal group, treatment was given from conception to parturition. In the postnatal group, treatment was given from parturition to 25 days postpartum. Hypothyroidism was induced by administration of 0.1% 6-n-propyl-2-thiouracil (PTU) in the drinking water of mothers. Body weights of the offspring were measured weekly. In each group, ten offspring were sacrificed at 120 days of age. Postnatal PTU treated pups showed delay in eye opening, teething, fur development, and weaning (35–37 days) compared to control animals (28–30 days). Body weight of offspring in the postnatal PTU treatment group was significantly decreased ($P < 0.001$), while the prenatal PTU treatment group showed a significant increase ($P < 0.0001$) compared to control animals. There was a significant ($P < 0.05$) reduction in paired ovarian weight of offspring in the postnatal PTU treatment group compared to control animals. Diameter of the ovaries was not affected by any treatment. Regarding the morphometery, only offspring in the prenatal PTU treatment group showed a significant ($P < 0.001$) increase in the diameter of graafian follicles. No significant difference was observed in morphometery of the granulosa layer, primary, and developing follicles of control and all treated groups. Number of primary, developing, and graafian follicles of all the treated groups was similar to that of the control group. The corpora lutea of the postnatal PTU treated group contained a population of large numbers of luteal cells compared to the control group. The prenatal PTU treated group did not exhibit a profound effect on ovarian morphology, histology, and morphometery. No difference was found in the serum estradiol concentration of control and PTU treated groups. *J. Exp. Zool. 293:407–413, 2002.* © 2002 Wiley-Liss, Inc.

It is a well-established fact that thyroid hormones play an important role in embryonic or foetal development of vertebrates (Sullivan et al., '87). Hypothyroidism and hyperthyroidism induces abnormalities in reproduction (Kalland et al., '78; Mattheij et al., '95). Hypothyroidism appears to delay sexual development in young males and toxic levels of thyroxin appear to impair reproductive function (Turner and Bagnara, '76). Hypothyroidism in rats results in fewer pregnancies and reduction in litter size (Varma et al., '78). Many of the young die due to insufficient lactation by mothers (Turner and Bagnara, '76).

Mattheij et al. ('95) induced hypothyroidism in female rats by thyroidectomy, and they found that ovaries of hypothyroid rats contain more large atretic follicles whose steroid metabolism was also disturbed. In addition, the disturbed steroid metabolism, both in the growing follicles and in the corpora lutea, caused the prolongation of luteal phase. They concluded that deficiency of thyroid hormone decreases the ovulation rate because hypothyroidism reduced the number of follicles that are able to ovulate.

Six-n-propyl-2-thiouracil (PTU) is a derivative of thiocarbamide (Turner and Bagnara, '76). It is an antithyroid (Yang and Gordon, '97), reversible goitrogen drug (Hardy et al., '96). It is effective in producing hypothyroidism early in life (Guedes and Pereira-Da-Silva, '93). PTU is transferred

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from mothers to their pups through the placenta (Marchant et al., '77; Mortimer et al., '97) and milk (Kawada et al., '88). 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., '92; Dijkstra et al., '96;Yang and Gorden, '97).

PTU is effective and safe in the treatment of hyperthyroidism during pregnancy in humans (Deborah et al., '94; Mortimer et al., '97).

Dijkstra et al. ('96) studied the effect of prepubertal hypothyroidism on ovarian development in rats by giving 0.1% PTU in the 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 animals. Corpora lutea were absent at day 40.

Chan and Ng ('95) induced hypothyroidism in mice by giving subcutaneous injection of 50 μg/g body weight PTU from postnatal day one onward. No difference was observed in ovarian histology between control and PTU treated mice at days 14, 21, and 28. There was a reduction in the number of graafian follicles and multilaminar follicles in PTU treated mice studied at postnatal days 14, 21, and 28 as compared to control animals. At postnatal day 28, Chan and Ng ('95) also found dead follicular cells around the oocyte, although the oocyte themselves appeared to be normal.

Different scientists studied the effect of PTU during the postnatal period of life and reported that the postnatal PTU treated pups have retarded growth and physical development, exhibit delay in eye opening and teething, are slow in responding to the general environment, and also have depressed body weight as compared to control animals (Meisami, '84; Tamasy et al., '84; Kawada et al., '88; Akaike et al., '91; Madeira et al., '91, '92; Akaike and Kato, '97).

MATERIALS AND METHODS

Mother Sprague-Dawely rats were randomly divided into three groups, and each group consisted of five rats.

Six-n-propyl-2-thiouracil (PTU, Sigma, St. Louis, MO) was used to induce hypothyroidism in mother rats. 0.1% PTU/day (0.1 g PTU dissolved in 95 ml distilled water) in drinking water was given to mothers. Five milliliters Rooh-Afza (Hamdard laboratories [WAQF], Lahore, Pakistan) was added to minimize the bitterness of PTU. One group of mothers (PTU1) received PTU from conception to parturition and their offspring received PTU through the placenta. Another group of mothers (PTU2) received PTU from parturition to 25 days postpartum and their offspring took PTU through milk.

The control group of mothers received tap water. Weekly feed, water consumption, and body weight of mothers were taken. The offspring from control and treated mothers were weaned (25–30 days), weighed, sexed, and housed four to five animals per cage. Weekly feed consumption and water uptake of the offspring from the three groups were recorded from weaning to the end of the experiment (120 days of age) by measuring the water remaining in drinking bottles and the remaining feed in the food box. Body weight (g) of pups was also recorded at weekly intervals.

Ten offspring from each group were anaesthetized and then sacrificed at 120 days of age to collect blood. Blood samples were collected for the estimation of estradiol (by radio immunoassay) from the aorta of the anaesthetized offspring. The ovaries were dissected out, ovarian size was taken by Vernier Calliper, and their weight was measured by Sarotoreious Digital Balance. Ocular micrometer was used at different magnifications for morphometeric analysis. Ovaries were fixed in sera fixative for histological examination.

Ovaries were sectioned at 5 μm thick and were stained with eosin and hematoxylin (McManus and Mowry, '60; Drury and Wallington, '80) for assessing the follicular development. For each ovary, at least ten sections were selected. The diameter and the total number of graffian, primary, and different ranges of developing follicles and their oocytes were taken. The diameter of the peripheral granulosa layer of graffian follicles was also measured.

Data of all three groups were expressed as mean $\pm$ SEM. In order to compare the control, pre-, and postnatal treatment groups, one way-ANOVA and Tukey's test were performed.

RESULTS

General observations

Mothers of prenatal PTU treated pups had a small litter size (5.40 $\pm$ 0.68) compared to

mothers of postnatal PTU treated pups (10.00 ± 2.04) and control mothers (10.33 ± 0.67).

Postnatal PTU treated pups started losing their weight as the treatment was given to their mothers. They showed: retarded physical development; delayed eye opening, teething, and fur development; and inattention to the general environment. These pups weaned later (35–37 days) than control pups (28–30 days).

Feed consumption, water uptake, and body weight (/week/individual) of control and treated mothers

Total mean feed consumption, water uptake, and body weight of mothers of prenatal treated pups (23.57 ± 5.55 g, 36.38 ± 7.34 ml, 226.0 ± 4.57 g) and mothers of postnatal treated pups (24.04 ± 1.31 g, 32.44 ± 3.61 ml, 219.20 ± 17.21 g) showed no significant difference compared to control mothers (27.97 ± 3.46 g, 36.72 ± 4.19 ml, 218.20 ± 12.55 g).

Results of offspring

Feed and water consumption of (/week/individual from weaning up to 120 days)

Pre- and postnatal PTU treated rats showed a significant ($P < 0.01$) reduction in total mean feed (54.07 ± 2.56 g, 75.52 ± 5.50 g) and water consumption (84.24 ± 5.51 ml, 83.5 ± 5.57 ml) compared to control animals (110.90 ± 5.582 g, 129.60 ± 6.68 ml) at 120 days of age.

Body weight (from birth up to 120 days)

Total mean body weight of prenatal PTU treated rats (155.20 ± 21.75 g) exhibited a highly significant ($P < 0.0001$) increase while postnatal PTU treated rats (109.10 ± 17.94 g) exhibited a highly significant ($P < 0.001$) decrease compared to controls (136.10 ± 20.10 g).

Ovarian weight

Paired ovarian weight of the prenatal PTU treatment group increased from control animals, but this variation is not appreciable. Paired ovarian weight of the postnatal PTU treatment group exhibited highly significant ($P < 0.001$) reduction compared to the paired ovarian weight of control and prenatal PTU treatment groups (Table 1).

TABLE 1. Total ovarian weight and diameter of control and PTU treated female offspring

Group	Total ovarian weight (mg)	Ovarian diameter (mm)	
		Right	Left
Control	73.50±2.67	6.12±0.15	6.24±0.19
PTU1	82.50±8.31b	5.93±0.13	5.89±0.16
PTU2	54.30±3.62a	5.44±0.22	5.19±0.16

[1]PTU, Prenatal treated rats; PTU2, Postnatal treated rats; a=treated group vs. control. b = prenatal vs. postnatal treatment group significantly ($P<0.001^{***}$) different from control by ANOVa and Tukey's test.

Ovarian diameter

Right and left ovarian diameters of the PTU treatment group decreased from control animals, but the difference is not significant (Table 1).

Histological observations

No difference was found in the structure of Graffian, primary, and developing follicles of control and PTU treated ovaries. Polyovular follicles were observed in the postnatal PTU treatment group. There is no difference in granulosa and thecal layer of control and PTU treated ovaries. It was noticed that the corpus luteum of the postnatal PTU treatment group had a remarkable increase in the number of large luteal cells and decrease in the number of small luteal cells compared to the control group.

Morphometeric analysis

Diameter of graffian follicles and oocytes

Diameters of graafian follicles of the prenatal PTU treatment group (585.40 ± 24.03 μm) exhibit a highly significant ($P < 0.001$) increase compared to diameters of control animals (414.80 ± 21.61 μm) and the postnatal PTU treatment group (396.00 ± 26.00 μm, Fig. 1). There was not a significant difference in the diameter of oocytes of the control and PTU treated groups.

Thickness of peripheral granulosa layer of graffian follicles

Thickness of the peripheral granulosa layers of pre- and postnatal PTU treatment groups exhibited nonsignificant difference as compared to the control group (Fig. 2).

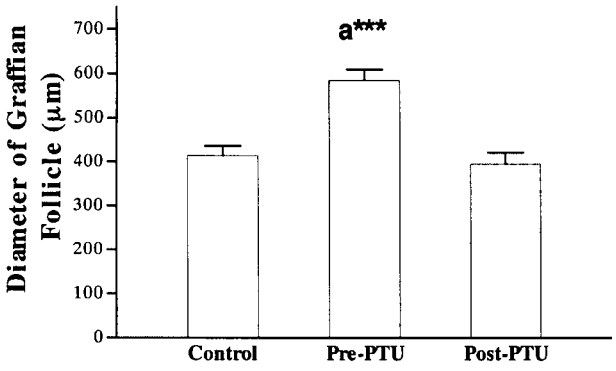

Fig. 1. Diameter of graffian follicles of prenatal PTU treatment group is significantly $P < 0.001$*** different from the control group by ANOVA and Tukey's test.

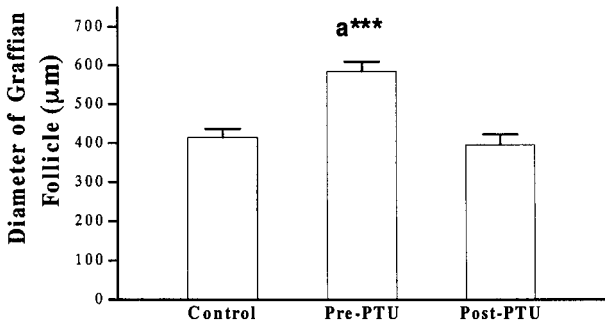

Fig. 3. Diameters of primary follicles of PTU treatment group are not significantly different from the control group by ANOVA and Tukey's test.

Diameter of primary follicles and oocytes

Diameter of primary follicles ranged from 50–100 μm. There was no significant difference in the mean diameter of primary follicles (Fig. 3) and their oocytes of control and PTU treatment groups.

Diameter of developing follicles and oocytes

Developing follicles are categorized into different groups (101–150, 151–200, 201–250, 251–300, 301–350 μm) with respect to the diameter. Mean diameter of different groups of follicles (Fig. 4) and their oocytes showed nonsignificant variation in control and PTU treatment groups.

Number of ovarian follicles

There was no appreciable difference in the mean number of graafian, primary, and developing follicles of control and PTU treatment groups (Table 2).

Serum estradiol

PTU treatment groups showed a slight reduction in serum estradiol levels compared to the control group although the reduction is statistically nonsignificant (Fig. 5).

DISCUSSION

In the present study, hypothyroidism was induced by giving 0.1% PTU in the drinking water of mothers, as also used by different investigators (Cooke et al., '92; Mendis-Handagama and Sharma, '94; Hardy et al., '96; Simorargkir et al., '97). It has been observed in the present study that mothers of prenatal PTU treated pups have a reduction in pregnancies and litter size. These findings are in accordance with the results reported by Varma et al. ('78) that maternal hypothyroidism resulted in fewer pregnancies and smaller litter size. In the

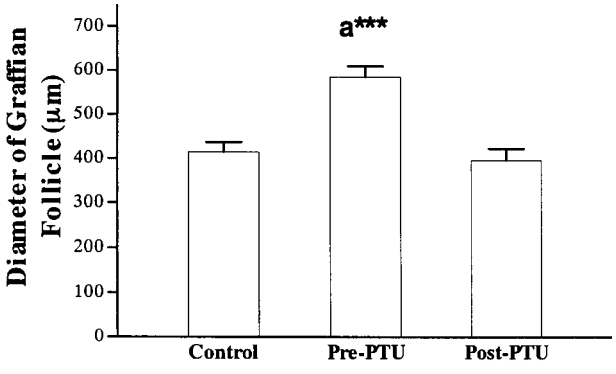

Fig. 2. Thickness of peripheral granulosa layer of PTU treatment group is not significantly different from the control group by ANOVA and Tukey's test.

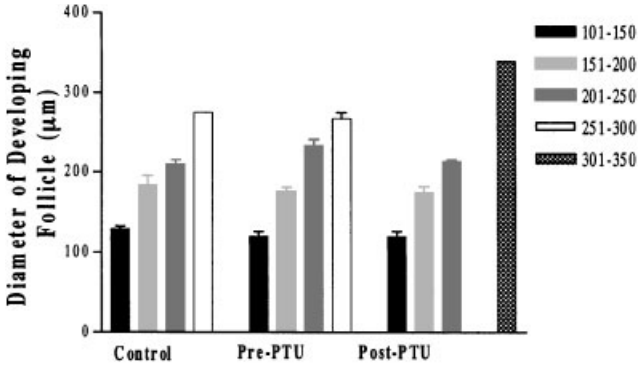

Fig. 4. Diameters of developing follicles of PTU treatment group are not significantly different from the control group by ANOVA and Tukey's test.

TABLE 2. Mean number of ovarian follicles of control and PTU treated offspring[1]

Follicles	Control	PTU1	PTU2
Gra-F	5.00±0.58	3.00±0.41	2.50±0.65
Pri-F	2.75±0.25	2.25±0.75	2.75±0.75
D101--150	3.25±0.96	2.50±0.87	1.50±0.50
D151--200	1.00±0.58	1.75±0.48	1.25±0.25
D201--250	1.00±0.41	1.00±0.41	0.50±0.29
D251--300	0.25±0.25	1.00±0.41	0.25±0.25
D301--350			0.25±0.25

[1]PTU1 prenatal PTU2 postnatal, Gra-F graffian follicles, Pri-F primary follicle, D different ranges of developing follicles, non significantly different from control by ANOVA and Tukey's test

present study, during the treatment period, mothers of prenatal PTU treated pups and mothers of postnatal PTU treated pups showed reduction in feed, water consumption, and body weight as compared to control animals. However, this decrease in feed, water consumption, and body weight of both groups of treated mothers showed nonsignificant difference at the end of the experiment. As the treatment was stopped, the treated mothers started taking sufficient amounts of feed and water.

In the present study, postnatal PTU treated pups had a low survival rate (30%). They also showed delay in eye opening, teething, fur development, growth, physical development, and weaning (35–37 days) as compared to control animals (28–30 days). It is possible that reduction in feed and water consumption of their mothers led to decreased milk production (containing sufficient amounts of PTU), which did not fulfil the nutritional requirements of these pups.

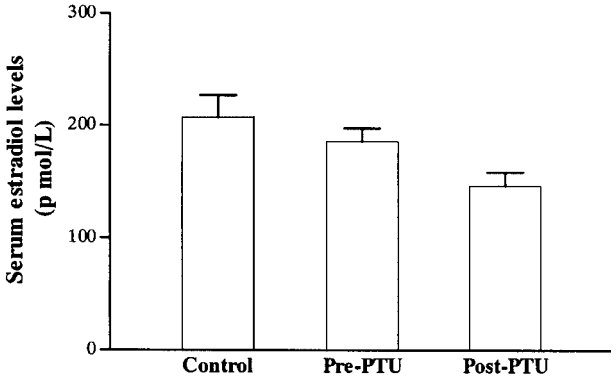

Fig. 5. Serum estradiol levels of PTU treatment group are not significantly different from the control group by ANOVA and Tukey's test.

Cooke et al. ('93) and Kawada et al. ('88) reported transfer of PTU through milk. Different scientists studied the effect of PTU in rats during the postnatal period of life and reported that these treated pups have: retarded growth and physical development; delay in eye opening, teething, and weaning; slowness of response to the general environment; and depressed body weight compared to control animals (Meisami, '84; Tamasy et al., '84; Blake et al., '85; Akaike et al., '91; Madeira et al., '91, '92; Madeira and Paula-Barbosa, '93; Akaike and Kato, '97).

It has also been noted in the present study that prenatal PTU treatment caused a slight but nonsignificant increase in the ovarian weight of offspring as compared to that of control animals. The consequences of hypothyroidism on the ovaries of prepubertal rats have recently been studied by Dijkstra et al. ('96) by giving 0.1% PTU in the drinking water of dams and pups from 0–40 days postpartum. Lower ovarian and body weight of hypothyroid rats was observed at 21 and 40 days of age. Decrease in ovarian and body weight of postnatal PTU treated hypothyroid rats has also been observed in the present study at 120 days of age. The results of the present and previous (Dijkstra et al., '96) study indicate that postnatal PTU treatment from birth onward results in lowered body weight and gonadal weight not only in prepubertal rats (40 days of age) but also in pubertal rats (120 days of age). It is concluded that transient postnatal PTU exposure causes proportional decreases in gonadal and body weight.

Regarding the number of ovarian follicles in 40 day old hypothyroid rats, Dijkstra et al. ('96) found that their ovaries contained 13.1% more secondary follicles with only two or three layers of granulosa cells and less antral follicles. Chan and Ng ('95) studied the effect of hypothyroidism induced by PTU (50 μg/g body weight) on the reproductive system of female mice at 14, 21, and 28 days postpartum. According to the results of their study, postnatal PTU treatment reduces the number of primordial, multilaminar, and graafian follicles at 14, 21, and 28 days of age. The present study revealed no change in the number of primary follicles of postnatal PTU treated offspring compared to control animals at 120 days of age. Whereas, small but statistically nonsignificant reductions have been found in the number of developing follicles and graafian follicles at 120 days of age compared to control animals. Mattheij et al. ('95) also reported that hypothyroidism

reduces the number of follicles which are able to ovulate.

The present study revealed no differences in the structures of primary, developing, and graafian follicles of offspring compared to control animals. However, polyovular follicles have been observed in the ovaries of the postnatal PTU treatment group. Peters ('78) has already observed polyovular follicles in normal rats. The development of polyovular follicles is not clear. They may be formed during the process of follicular organization. It could be possible that they represent some phenomenon of follicular atresia or that deficiency of estrogen is responsible for their development (Peters, '78). No difference was found in the mean diameters of various classes of secondary follicles and their oocytes of postnatal PTU treated rats at 40 days of age compared to control animals (Dijkstra et al., '96). Results of the present study correlate to this finding in the sense that postnatal PTU treatment has no effect on the diameter of developing follicles and their oocytes at 120 days of age compared to control animals. Also, no effect has been found on the diameter of primary follicles.

It was reported by Greenwald ('78) that, in normal rats, follicles having diameters larger than 400 μm have an antral cavity. In the current study, the diameter of graafian follicles in control ovary is found to be 414.80 μm, which corresponds to this finding. Dijkstra et al. ('96) noticed increased diameters of advanced antral follicles in postnatal PTU treated rats at 40 days of age compared to control animals. The present study finds that prenatal PTU treatment caused a significant increase ($P < 0.001$) in diameter of graafian follicle, while postnatal PTU treatment caused a slight but nonsignificant reduction in diameter compared to control animals.

The present study revealed that graafian follicles of the prenatal PTU treatment group exhibit a slight but nonsignificant increase in the thickness of their peripheral layers of granulosa cells compared to control animals. Whereas, postnatal PTU treatment caused a small but nonsignificant decrease in the thickness of the peripheral granulosa layer as compared to control animals. From this result, it can be suggested that the thickness of the 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, '95).

The current study revealed an increase in body weight, ovarian weight, and diameter of graafian follicles of prenatal PTU treated offspring compared to control animals. Conversely, postnatal PTU treated offspring showed reduction in body weight, ovarian weight, and diameter of graafian follicles compared to control animals. This result corresponds to the finding of Espey ('78) that, in a given species, the size of mature graafian follicles is proportional to the body weight of the female.

In the present study, the corpora lutea of postnatal PTU treated offspring contained a higher population of large luteal cells as compared to control animals. The presence of luteal cells is indicative of steroidogenic activity of corpora lutea (Niswender and Nett, '94). In the present study, the concentration of serum estradiol shows no difference among control and PTU treatment groups. Although offspring of the postnatal PTU treatment group had a small decrease in serum estradiol concentration as compared to control animals, this decrease is statistically nonsignificant. In control and PTU treated groups, there was no difference in the number of ovarian follicles and the thickness of the peripheral layer of granulosa cells. Finally, it is possible that PTU does 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, '95). Hence, there is no significant difference in the serum estradiol concentration of control and PTU treatment groups.

In summary, it is concluded from the present study that the administration of 0.1% PTU to immature female offspring from 0–25 days postpartum affects growth and physical development, and causes a decrease in body weight and ovarian weight at 120 days of age. This indicates the importance of thyroid hormone during the early postnatal period of life regarding growth, physical development, and ovarian weight. Prenatal PTU treatment has no profound effect on ovarian morphology, histology, and morphometery of offspring. Treatments during the pre- and postnatal periods of life do not alter the concentration of estradiol in offspring at 120 days of age.

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