Transient Induction of Polycystic Ovary-Like Syndrome in Immature Hypothyroid Rats (44319)

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Abstract. **Hypothyroidism in the human female is often associated with ovarian follicular cysts and hyperandrogenism, two cardinal signs of polycystic ovary syndrome. To explore the intraovarian changes that lead to follicular cyst formation in hypothyroidism, we have created a prepubertal hypothyroid rat model. These hypothyroid rats are hyperandrogenic and develop transient ovarian follicular cysts. Hypothyroidism in newborn rats was induced by providing the lactating dams with 0.04% propylthiouracil (PTU)-containing water. Subsequently, female rats were weaned and kept on PTU-containing water. On Day 25 of age, the rats were primed with 15 international units of pregnant mare's serum gonadotropin (PMSG) in 100 μl of phosphate buffered saline. Two days later, to initiate pseudopregnancy, they were injected with five international units of human chorionic gonadotropin (hCG). The animals were sacrificed at appropriate times, and blood and ovaries were collected for analyses. Control experiments were done with euthyroid rats. Two days after PMSG injection, well-developed antral follicles were observed in both the hypothyroid and euthyroid rats. Two days after hCG injection, while the euthyroid rat ovaries, as expected, contained numerous corpora lutea (CL), the hypothyroid rat ovaries still retained antral follicles. Some of these follicles with degenerating oocytes showed signs of luteinization. By 3–4 days post-hCG injection, the hypothyroid rat ovaries developed cystic follicles. By Day 6, however, the hypothyroid rat ovaries were indistinguishable from those of the euthyroid rats. Although serum testosterone concentrations were significantly elevated in the hypothyroid rats on Days 1–3, progesterone concentrations were not significantly different from the euthyroid animals. However, by Days 8–14, the hypothyroid rats had significantly higher serum progesterone concentrations. This model will be useful for investigating the intraovarian biochemical changes that lead to follicular cyst development in response to acute gonadotropin treatment.** [P.S.E.B.M. 1998, Vol 219]

In 1935 Stein and Leventhal (1) described the polycystic ovary syndrome (PCOS) as a disease associated with hirsutism, amenorrhea, and infertility. Since then, a number of other conditions, including elevated luteinizing hormone (LH) concentrations, hyperinsulinemia, hyperandrogenism, and elevated 17α-hydroxyprogesterone have come to be associated with PCOS (Refs. 2, 3, and references therein). Thus, at present, with the exception of ultrasonographic criteria, no single accepted definition of PCOS exists. However, the recognition of multiple disorders in PCOS has led to the establishment of many animal models to study the underlying events in the development of the polycystic ovary (4–10). These models have been extremely useful to our present understanding of the complex nature of the development of cystic follicles.

For several years, we have used the adult hypothyroid rat model (10) to elucidate the hormonal changes associated with the development of follicular cysts (11–13). Our interest in this animal model stems from the fact that cystic follicle–containing ovaries in humans are often associated with hypothyroidism (14–26). Since the availability of ultrasonography, the association between hypothyroidism and

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ovarian cysts has become increasingly evident, and the presence of cysts now has been considered a diagnostic marker for hypothyroidism (20–22). Further, the association between hypothyroidism and ovarian cyst development is more than a correlation since thyroid hormone replacement therapy results in the resolution of the cysts in both humans (17–20, 26) and rats (10). Furthermore, elevated serum concentrations of luteinizing hormone (LH), androgen, estradiol, and prolactin observed in the hypothyroid rat model (11–13, 27), are also observed in juvenile (17, 18, 26) and adult hypothyroid patients (25, 28, 29).

It has been recognized for some time that the vasculature must be the major source of fluid in the cysts (Ref. 30 and references therein). Thus, alterations in the ovarian vasculature probably precede fluid accumulation in the cysts. Since the induction of cysts in the present hypothyroid model requires chronic administration of hCG to the rats (10), we wanted to develop an animal model where cysts could be induced in response to an acute hormonal stimulus so we could investigate the early intraovarian changes that precede cyst development. Toward this goal, in this study, we have combined the hypothyroid rat model (10) and immature pseudopregnant rat model (31), to induce transient follicular cysts in the immature rat ovary. In this model, a single injection of PMSG followed 2 days later by a single injection of hCG to hypothyroid immature rats causes development of follicular cysts. We believe that this model will be useful for studying changes in various intraovarian biochemical parameters that are associated with the morphogenesis of follicular cysts.

Materials and Methods

Hypothyroid Animal Model. All of the experiments described in this study conform to *The Guide for the Care and Use of Laboratory Animals* published by the National Research Council (Publications No. 0-309-05377-3, 1996) and were approved by the Kent State University Animal Care and Use Committee.

Male and cycling female Long Evans rats were maintained on a regulated photoperiod (12:12-light:dark cycle) and temperature (24°C) and with standard rodent chow (Prolabs, St. Louis, MO) and water available *ad libitum.* Immature female rats for experiments were produced by breeding these rats as needed. Hypothyroidism in newborn rats was induced by providing the lactating dams with 0.04% PTU (Sigma Chemical Company, St. Louis, MO)-containing water. Subsequently, the female rats were weaned after 21 days and kept on PTU-containing water until termination of the experiment. On Day 25 of age, the rats were weighed and primed with 15 international units (IU) of PMSG (Sigma) in 100 μl of phosphate buffered saline (PBS). Two days later, pseudopregnancy was initiated by injecting the rats with 5 IU of hCG (Sigma) in 100 μl of PBS as described previously (32). Control euthyroid rats were also treated as above without exposure to PTU water. At appropriate times, the animals were weighed and sacrificed, and the blood and ovaries were collected for analyses. At least three animals were used at each time point.

Tissue Processing, Hormone Assays and Statistical Analysis. For histology, the ovaries were fixed overnight in Bouin's fixative and processed for paraffin embedding. Sections were cut at 6 μm and stained with hematoxylin and eosin. Serum progesterone and testosterone were measured directly with the ACS:180 automated chemiluminescence system (Chiron Diagnostics, Walpole, MA), and the values were analyzed by one-way analysis of variance (ANOVA) followed by Scheffe's F test. Significance was determined at $P < 0.05$. Results are expressed as mean ± standard error of the mean (SEM).

Results

Effects of Hypothyroidism on Body and Ovarian Weights. The body weights of PTU-treated hypothyroid rats, as expected, were significantly lower than the control euthyroid rats on the day of PMSG injection (hypothyroid rats, 33.5 ± 0.7 g; control rats, 66.7 ± 2.2 g). Further, lower body weights were maintained throughout pseudopregnancy in the hypothyroid rats (Fig. 1). The marked rise in the weights of the euthyroid rats on Day 14 of pseudopregnancy presumably reflects the growth associated with pubertal maturation.

Although the ovarian weights of both the hypothyroid and the euthyroid rats were essentially the same on the day of PMSG injection (hypothyroid rats, 12.4 ± 1.2 mg/pair; control rats, 12.0 ± 1.8 mg/pair), by the first day of pseudopregnancy (one day after hCG injection) the ovarian weights of the hypothyroid rats were significantly lower

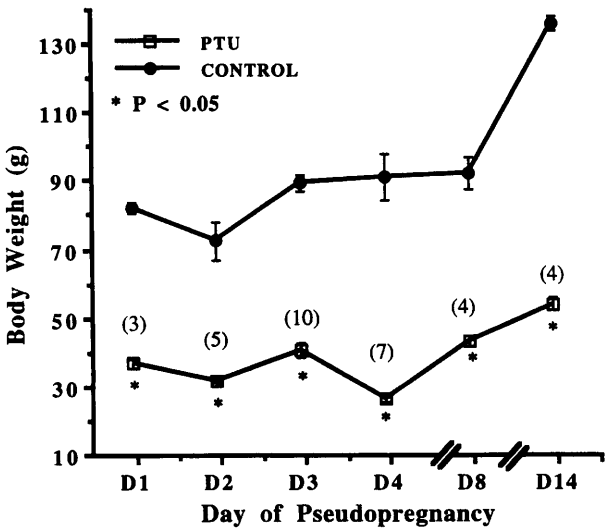

Figure 1. Body weights of euthyroid (control) and hypothyroid (PTU) rats. Throughout the experimental period, as expected, the body weights of the hypothyroid rats were significantly lower than those of the euthyroid rats. The marked rise in the weights of the euthyroid rats on Day 14 of pseudopregnancy likely reflects the growth associated with pubertal maturation. Values are mean ± SEM. The number of animals at each time point is shown in parentheses.

than those of the euthyroid rats (Fig. 2). However, by the third and fourth days of pseudopregnancy, the hypothyroid rat ovarian weights were significantly higher than those of the euthyroids (Fig 2). By the middle of pseudopregnancy, there were no significant weight differences between the two groups (Fig 2).

Effects of PMSG alone on Ovarian Morphology. Examination of the ovaries 2 days after PMSG injection showed fully developed antral follicles in both the euthyroid (Fig. 3A) and the hypothyroid rats (Fig. 3B). The ovarian interstitium, however, appeared to be more compact in the hypothyroid rats (Fig. 3B) whereas the interstitial tissue was more dispersed in the stromal tissue. Further, a few collapsed follicles were also observed in the hypothyroid rat ovaries (Fig. 3B).

Ovarian Morphology During Pseudopregnancy. When the euthyroid rat ovaries were examined 2 days after hCG injection (Day 2 of pseudopregnancy), as anticipated, many CL were observed (Fig. 4A). On the other hand, the hypothyroid rat ovaries were devoid of CL and contained many antral follicles (Fig. 4B). In addition, the oocytes in many of these follicles were fragmented, and the follicles showed signs of luteinization (Figs. 4C & D), which could potentially give rise to corpora atretica. Occasionally, polyovular follicles (follicles with more than one oocyte) were also observed in the hypothyroid rat ovaries (not shown). By the third and fourth days of pseudopregnancy, the ovaries in many of the hypothyroid rats contained mostly cystic follicles with a few layers of granulosa cells (Fig. 5A). The size of the cysts ranged from about 1 to 1.2 mm in diameter (data not shown). Note that the term pseudopregnancy in the hypothyroid rats here refers not so much to the classical definition of postovulatory pseudopregnancy but rather to the period following hCG injection.

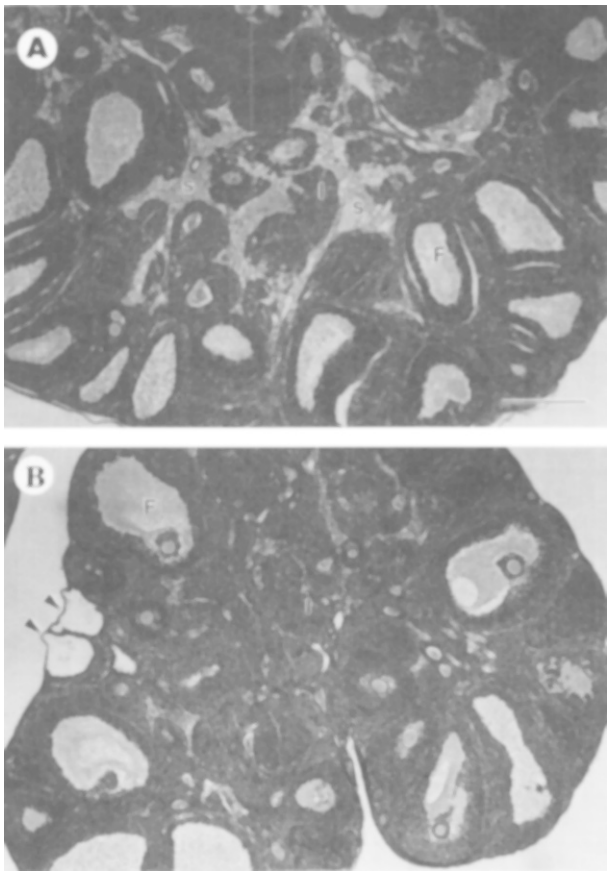

Figure 3. Photomicrographs of (A) PMSG primed euthyroid and (B) hypothyroid rat ovaries. Note the presence of large antral follicles, F, in both groups. The interstitial glands, I, appear to be more compact in the hypothyroid ovary (B) than in the euthyroid ovary (A) where the interstitial tissue is dispersed in the prominent stroma, S. Arrowheads indicate two collapsed follicles. Scale bar = 500 μm.

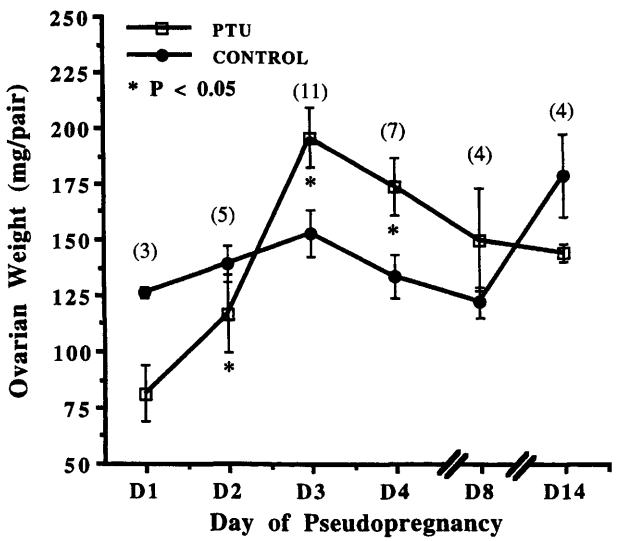

Figure 2. Ovarian weights of euthyroid (control) and hypothyroid (PTU) rats. One day after hCG injection (Day 1 of pseudopregnancy), the ovarian weights of the hypothyroid rats were significantly lower than those of the euthyroid rats. On Days 3 and 4, however, the hypothyroid ovarian weights were higher than those of the euthyroid ovaries. By the middle of pseudopregnancy, there were no significant weight differences between the two groups. Values are mean ± SEM. The number of animals at each time point is shown in parentheses.

Where luteinization occurred (Fig. 5B), prominent neovascularization zones were observed (Figs. 5C & D). By the eighth day of pseudopregnancy, however, no morphological distinctions could be made between the euthyroid and the hypothyroid rat ovaries. Both contained what appeared to be normal healthy CL. The average size of the CL in both groups was about 1 mm in diameter (not shown). By the fourteenth day of pseudopregnancy, as expected, the CL of the euthyroid rats had involuted, and their luteal cell nuclei were pyknotic (Fig. 6A). Remarkably, the luteal cells of the hypothyroid rat ovaries appeared healthy with a large cytoplasmic to nuclear ratio and euchromatic nuclei (Fig. 6B).

Testosterone and Progesterone Concentrations During Pseudopregnancy. Two days after PMSG injection, serum testosterone concentrations were not different between the hypothyroid and the euthyroid rats. However, in response to hCG injection, serum testosterone concentrations significantly decreased in the euthyroid rats (Fig. 7). The decrease in serum testosterone in these animals may reflect the differentiation of thecal cells

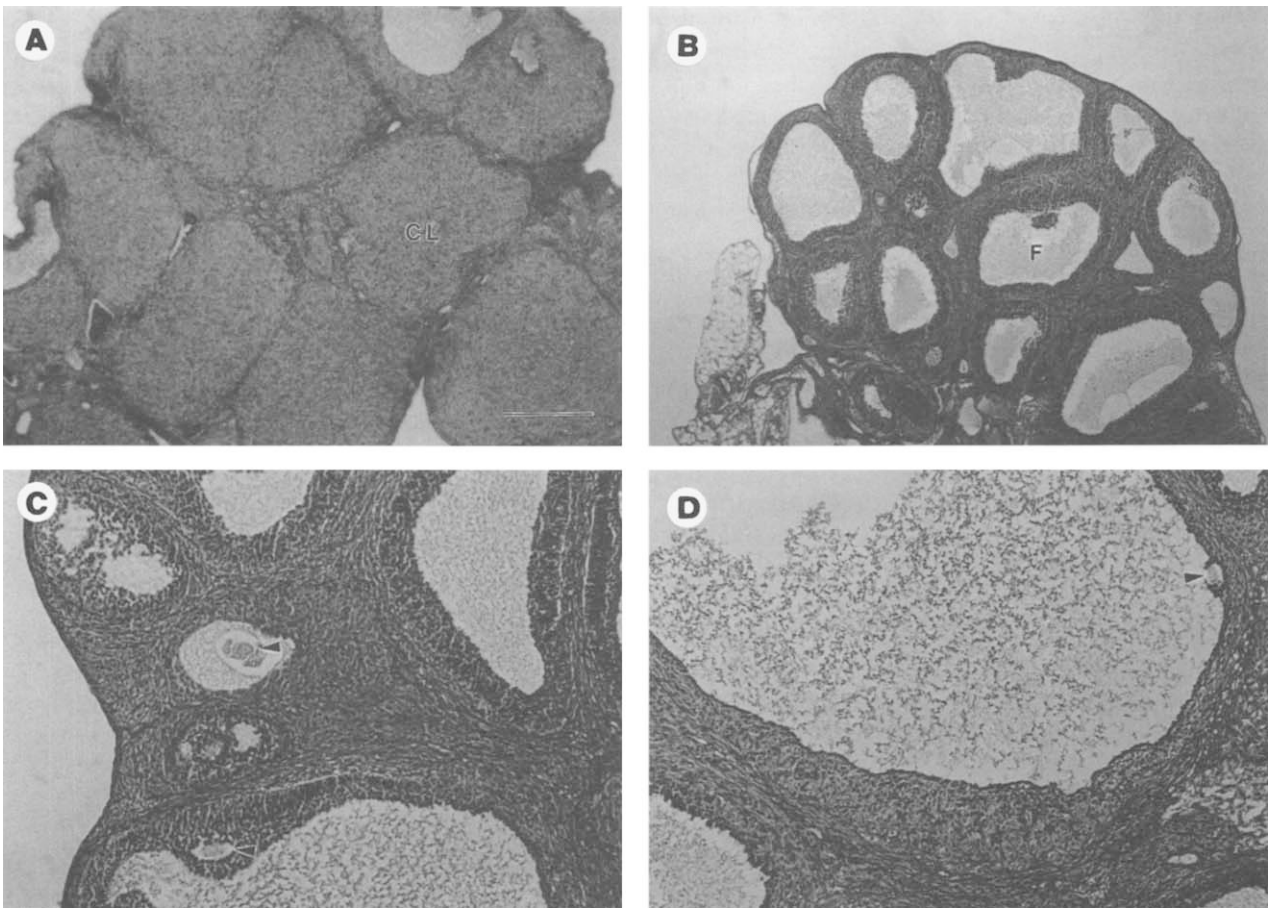

Figure 4. Photomicrographs of ovaries from Day 2 pseudopregnant rats. Nearly fully developed corpora lutea, CL, are seen in the euthyroid ovary (A). In contrast, the hypothyroid ovary (B) is filled with many antral follicles, F. Degenerating oocytes (arrowheads) are present in the hypothyroid ovaries (C and D). Figs. A and B: Bar = 500 μm. Figs. C and D: Scale bar = 200 μm.

into luteal cells in the euthyroid ovaries. In contrast, serum testosterone concentrations were maintained at pre-hCG injection values through the fourth day after the injection of hCG in the hypothyroid rats (Fig. 7). During the first three days of pseudopregnancy, serum testosterone concentrations were significantly higher in the hypothyroid rats than in the euthyroid rats (Fig. 7). By Day 4, no significant difference was observed between the two groups. By Day 8, testosterone concentrations had reached the basal level in both groups. Serum progesterone concentrations were similar in both the euthyroid and the hypothyroid rats until Day 4 of pseudopregnancy (Fig. 8). However, after Day 4, serum progesterone was significantly higher in the hypothyroid rats than in the euthyroid rats. By Day 14, as expected (31), serum progesterone declined close to its basal value in the euthyroid rats. To the contrary, serum progesterone concentration remained significantly elevated in the hypothyroid rats.

Discussion

We have established an immature hypothyroid model in which PMSG-primed rats developed ovarian cystic follicles in response to a single injection of hCG. Although we did not measure the serum concentrations of thyroid hormones and TSH in our model, it is clear that treatment of male neonatal rats with PTU from birth to Day 25 *post partum* severely decreases the serum thyroid hormone levels (33) while elevating TSH levels (34). Also in the adult hypothyroid rat model thyroid hormones are decreased, and TSH levels are elevated in response to PTU treatment (35).

In this model, transient cystic follicles (Fig. 5A) and high serum androgen levels (Fig. 7) were observed in response to a single injection of hCG to PMSG-primed rats. These cysts (<1.5 mm) are similar to the small cysts we have observed in the adult hypothyroid rats after three injections of hCG (13) but are smaller than the 1.8 mm cysts observed after 10 days of hCG treatment (10). Unlike the euthyroid rats, where prompt luteinization was observed in response to hCG, the hypothyroid rat ovaries showed many cystic follicles as late as 4 days after hCG injection (Fig. 5A). However, by Day 8 (figure not shown), no cystic follicles were observed in these ovaries. Instead, the ovaries were full of CL. It is not clear if any of these were true CL from ovulated follicles although a developing CL observed on Day 4 of pseudopregnancy (Fig. 5B) appears to be from an ovulated follicle. However, it is difficult to rule out the possibility that CL could also arise from unovulated fol-

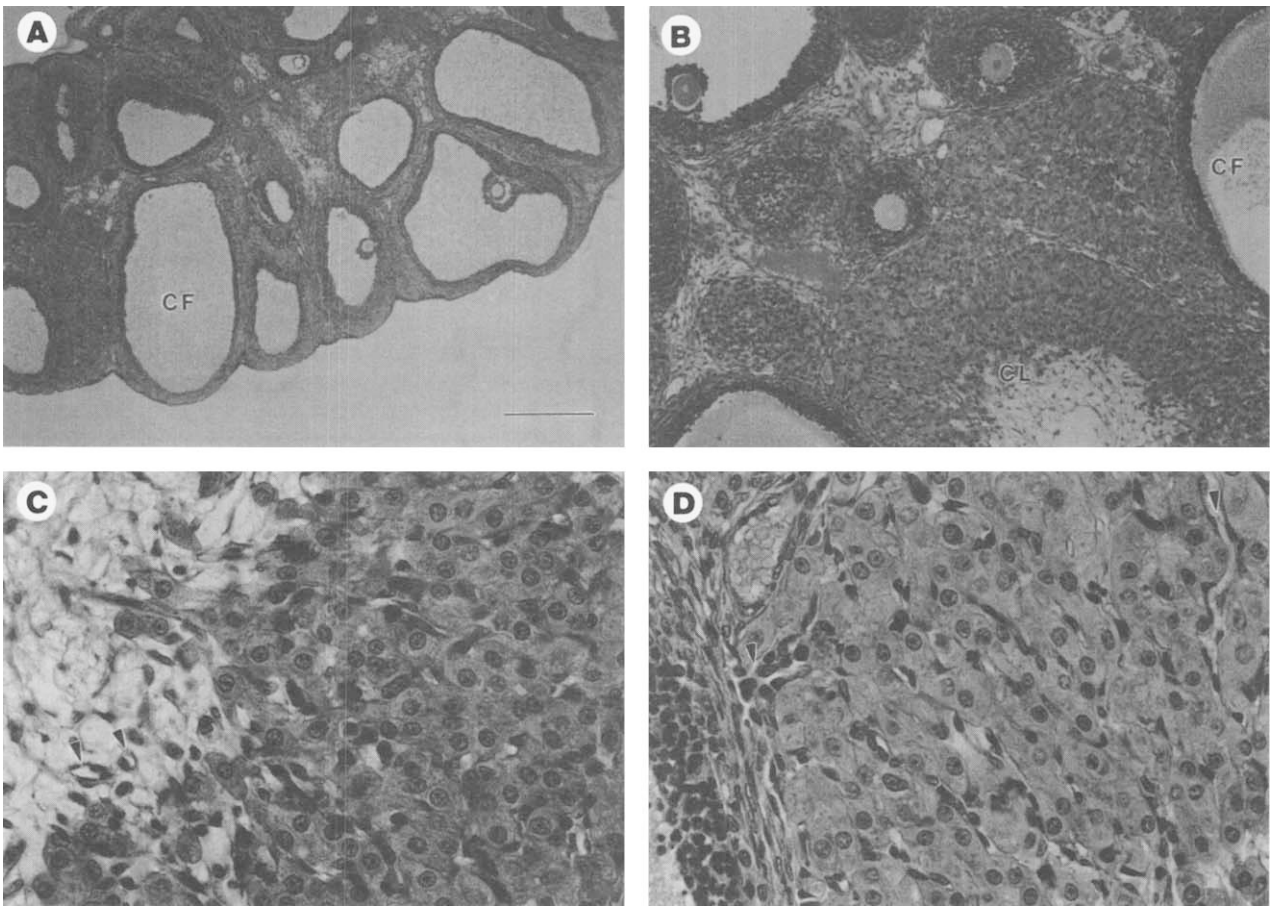

Figure 5. Photomicrographs of ovaries from Day 4 pseudopregnant hypothyroid rats. Many cystic follicles, CF, are seen in a hypothyroid ovary (A). Some ovaries also contain developing corpus luteum, CL (B). Luteinization is associated with prominent neovascularization (C and D) zones (arrowheads). Fig. A: Bar = 500 μm; Fig. B: Bar = 200 μm. Figs. C and D: Scale bar = 50 μm.

licles since luteinization was present in follicles with degenerating oocytes (Fig. 4D). Moreover, hypothyroidism appears to be an important contributing factor in inhibiting ovulation since clomiphene does not induce ovulation in hypothyroid women (36). This may result from impaired follicular development in the hypothyroid ovary. It is interesting to note that in neonatal hypothyroid male rats, testicular maturation of both germ cells (37) and Sertoli cells (38) was delayed. Thyroid hormone normally inhibits neonatal Sertoli cell proliferation and stimulates their differentiation (39). Thus, in the absence of thyroid hormones, the Sertoli cells, counterparts of granulosa cells, may continue to proliferate rather than differentiate. Further, it has been proposed that the increased Sertoli cell proliferation in the neonatal hypothyroid rats might be due to the rising serum TSH concentration (34). Since TSH is able to bind to and activate both FSH (40) and hCG receptors (41), a simultaneous decrease in serum thyroid hormones and rise in TSH in the hypothyroid female rats (35) may also lead to potential defects in follicular maturation.

An ovulatory defect is observed even in euthyroid animals with cystic (42, 43) and noncystic ovaries (44). Further, in the anovulatory transgenic mice with elevated serum LH, luteinized cystic follicles and true CL-like structures have been observed (45). In addition, in the rat, luteal tissue derived from cystic or atretic follicles is difficult to distinguish from that derived from the normal CL (46). Therefore, the assignment of the origin of CL in the hypothyroid rat ovary requires future experiments involving thecal- and granulosa cell–specific lectins or immunological markers.

The increased ovarian sensitivity to gonadotropins, as discerned by the increased ovarian weight (Fig. 2) and serum sex steroids (Fig. 7, 8), has been observed previously (10–13, 47), and this increased sensitivity is diminished in response to thyroxine replacement (48). An increase in the number of hCG (49) and FSH (50) receptors in the hypothyroid ovary may account in part for this ovarian hypersensitivity. Additionally, the increased thyrotropin (TSH) in the hypothyroid rats may synergize with the actions of gonadotropins through their receptors (41). TSH also has been shown to increase progesterone production by rat luteal cells from both hypothyroid and euthyroid rats *in vitro* (51). Thus, the maintained concentrations of serum testosterone and progesterone (Fig. 8) may well reflect the combined responsiveness of the ovaries to both endogenous gonadotropins and TSH.

The high serum concentration of progesterone on Day 14 in the hypothyroid rats could result from the apparent

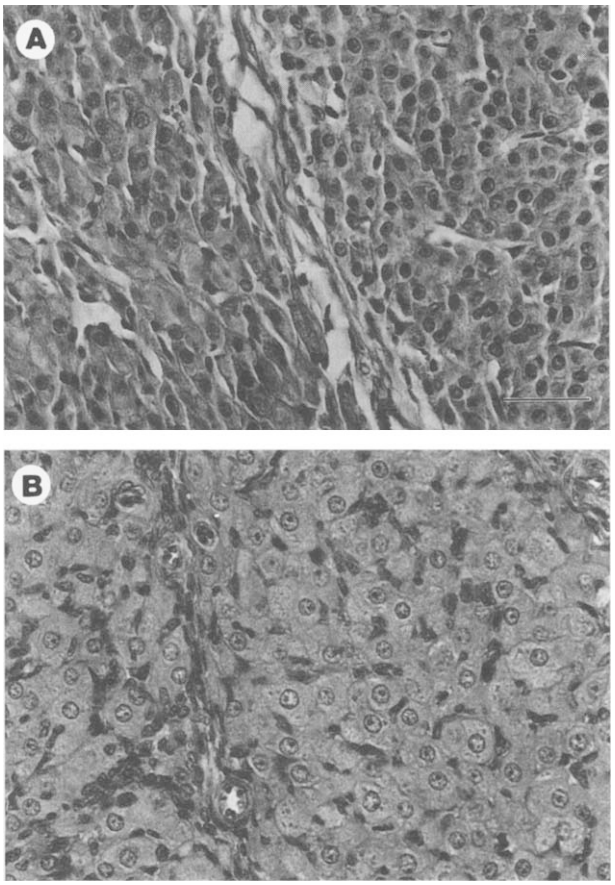

Figure 6. Photomicrographs of corpora lutea, CL, from Day 14 pseudopregnant euthyroid (A) and hypothyroid (B) rats. It can be clearly observed that the luteal cells of the euthyroid CL (A) have a small cytoplasmic to nuclear ratio in comparison to that of the hypothyroid CL (B). The euthyroid luteal cell nuclei also appear to be pyknotic. Scale bar = 50 μm.

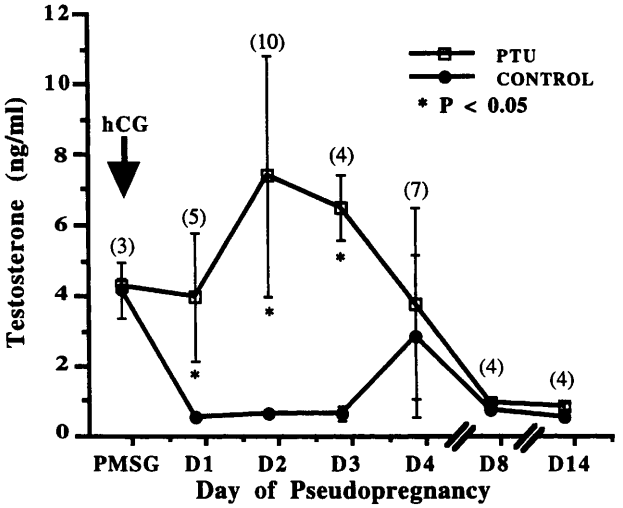

Figure 7. Serum testosterone in euthyroid (control) and hypothyroid (PTU) rats. Serum testosterone concentrations were not different between the hypothyroid and euthyroid rats 2 days after PMSG injection (PMSG = Day 0 of pseudopregnancy). However, in response to hCG injection, the serum testosterone concentrations were significantly decreased in the euthyroid rats than in the hypothyroid rats during the first 3 days of pseudopregnancy. By Day 4, no significant difference was observed between the two groups. By Day 8, testosterone concentrations had reached the basal levels in both groups. Values are mean ± SEM. The number of animals at each time point is shown in parentheses.

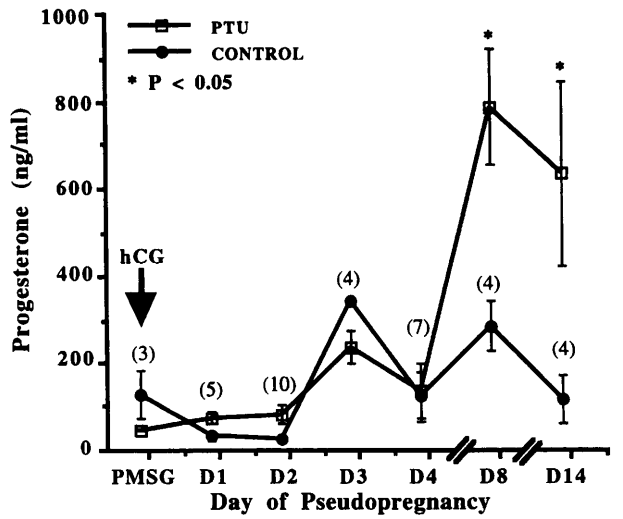

Figure 8. Serum progesterone in euthyroid (control) and hypothyroid (PTU) rats. Serum progesterone concentrations were also not different between the hypothyroid and euthyroid rats 2 days after PMSG injection (PMSG = Day 0 of pseudopregnancy). During the first 4 days of pseudopregnancy, no significant differences were observed between the two groups. Subsequently, however, serum progesterone concentrations were significantly higher in the hypothyroid rats than in the euthyroid rats. Values are mean ± SEM. The number of animals at each time point is shown in parentheses.

extended life span of the luteal tissue if the cells continue to show increased sensitivity to gonadotropins for the reasons we have discussed above. In this context it is interesting to note that prolonging the half-life of luteinizing hormone in transgenic mice not only leads to the development of cystic follicles but also prolongs the length of pseudopregnancy (52). Alternatively, delayed luteinization of the unruptured follicles in the hypothyroid ovary could also account for the elevated serum progesterone in late pseudopregnancy. Whether one or both of these above possibilities takes place in our model remains to be determined.

The intraovarian mechanisms that lead to the development of cystic follicles have not been studied extensively. Recently, however, it has been shown (53) that polycystic ovarian follicles stained strongly for vascular permeability factor/vascular endothelial growth factor (VPF/VEGF). VPF/VEGF causes both fluid accumulation (54) and angiogenesis (55). Hypoxia is a potent inducer of VPF/VEGF (56). It has been observed that polycystic condition is associated with increased lactate accumulation (57), an indicator of anaerobic tissue metabolism. Thus, fluid accumulation in the cystic follicles could be mediated by VPF/VEGF. However, the regulation of VPF/VEGF or other factors that contribute to cyst development remains to be explored. The model developed in this study will be especially useful for exploring the changes in the ovarian vasculature associated with cyst development since the neonatal ovaries contain neither ovulatory follicles nor corpora lutea prior to exogenous hormone treatment.

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1. Stein IF, Leventhal ML. Amenorrhea associated with bilateral polycystic ovaries. Am J Obstet Gynecol **29:**181–191, 1935.
2. Udoff L, Adashi EY. Polycystic ovarian disease: A new look at an old subject. Curr Opin Obstet Gynecol **7:**340–343, 1995.
3. Franks S. The ubiquitous polycystic ovary. J Endocrinol **129:**317–319, 1991.
4. Brawer JR, Naftolin F, Martin J, Sonnenschein C. Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. Endocrinology **103:**501–512, 1978.
5. McCarthy GF, Brawer JR. Induction of Stein-Leventhal-like polycystic ovaries (PCO) in the rat: A new model for cystic ovarian disease. Anat Rec **228:**137–144, 1990.
6. Barraclough CS. Modification in the CNS regulation of reproduction after exposure of prepubertal rats to steroid hormones. Recent Prog Horm Res **22:**503–515, 1966.
7. Mahesh VB, Mills TM, Bagnell CA, Conway BA. Animal models for study of polycystic ovaries and ovarian atresia. Adv Exp Med Biol **219:**237–257, 1987.
8. Bogovich K. Animal models for polycystic ovary syndrome. In: Dunaif A, Givens JR, Haseltine FP, Merriam GR, Eds. Current Issues in Endocrinology and Metabolism-Polycystic Ovary Syndrome. Boston: Blackwell Scientific Publications: pp129–140, 1987.
9. Poretsky L, Clemons J, Bogovich K. Hyperinsulinemia and human chorionic gonadotropin synergistically promote the growth of ovarian follicular cysts in rats. Metabolism **41:**903–910, 1992.
10. Leathem JH. Hormonal influences on the gonadotropin-sensitive hypothyroid rat ovary. Anat Rec **131:**487–499, 1958.
11. Bruot, BC. Hormone secretion by euthyroid and hypothyroid rat ovaries during the early stages of hCG-induced ovarian cyst development. Proc Soc Exp Biol Med **184:**206–210, 1987.
12. Lee MT, Adams WC, Bruot BC. Circulating hormone concentrations in hypothyroid rats with induced polycystic ovaries. Proc Soc Exp Biol Med **198:**737–741, 1991.
13. Takacs-Jarret M, Bruot BC. Steroid secretion by follicles and cysts from the hypothyroid, hCG-treated rat. Proc Soc Exp Biol Med **207:**62–66, 1994.
14. Van Wyk J, Grumbach M. Syndrome of precocious menstruation and galactorrhea in juvenile hypothyroidism: An example of hormonal overlap in pituitary feedback. J Pediatr **57:**416–435, 1960.
15. Van Gelderen HH. Precocious menstruation in hypothyroidism. Arch Dis Child **37:**337–339, 1962.
16. Hayles AB, Cloutier MD. Clinical hypothyroidism in the young: A second look. Med Clin North Am **56:**871–884, 1972.
17. Barnes ND, Hayles AB, Ryan RJ. Sexual maturation in juvenile hypothyroidism. Mayo Clin Proc **48:**849–856, 1973.
18. Lindsay A, Voorhess M, MacGillivray M. Multicystic ovaries detected by sonography in children with hypothyroidism. Am J Dis Child **134:**588–592, 1980.
19. Evers JLH, Rolland R. Primary hypothyroidism and ovarian activity: Evidence for an overlap in the synthesis of pituitary glycoproteins. Case report. Br J Obstet Gynecol **88:**195–202, 1981.
20. Riddlesberger MM, Kuhn JP, Munschauer RW. The association of juvenile hypothyroidism and cystic ovaries. Radiology **139:**77–80, 1981.
21. Jafri SZH, Bree RL, Silver TM, Ouimette M. Fetal ovarian cysts: Sonographic detection and association with hypothyroidism. Radiology **150:**809–812, 1984.
22. Lyon AJ, De Bruyn R, Grant DB. Transient sexual precocity and ovarian cysts. Arch Dis Child **60:**819–822, 1985.
23. Nishi Y, Masuda H, Iwamori H, Urabe T, Sakoda K, Uozumi T, Usui T. Primary hypothyroidism associated with pituitary enlargement, slipped capital femoral epiphysis, and cystic ovaries. Eur J Pediatr **143:**216–219, 1985.
24. Pringle PJ, Stanhope R, Hindmarsh P, Brook CGD. Abnormal pubertal development in primary hypothyroidism. Clin Endocrinol **28:**479–486, 1988.
25. Ghosh S, Kabir SN, Pakrashi A, Chatterjee S, Chakravarty B. Subclinical hypothyroidism: A determinant of polycystic ovary syndrome. Horm Res **39:**61–66, 1993.
26. Hansen KA, Tho SPT, Hanly M, Path MRC, Moretuzzo RW, McDonough PG. Massive ovarian enlargement in primary hypothyroidism. Fertil Steril **67:**169–171, 1997.
27. Lee MT, Bruot BC, Adams WC. Hormonal changes during the early development of ovarian cysts in the rat. Biol Reprod **35:**542–548, 1986.
28. Keye WR, Yuen BH, Knopf RF, Jaffe RB. Amenorrhea, hyperprolactinemia, and pituitary enlargement secondary to primary hypothyroidism. Obstet Gynecol **48:**697–702, 1976.
29. Shahshahani MN, Wong ET. Primary hypothyroidism, amenorrhea, and galactorrhea. Arch Intern Med **138:**1411–1412, 1978.
30. Delson B. Non-neoplastic ovarian cysts: Their relation to spiral arteries in the human ovary. Am J Obstet Gynecol **57:**1120–1132, 1949.
31. Horikoshi H, Wiest WG. Interrelationships between estrogen and progesterone secretion and trauma-induced deciduomata: On causes of uterine refractoriness in the "Parlow Rat." Endocrinology **89:**807–817, 1971.
32. Reich R, Miskin R, Tsafriri A. Follicular plasminogen activator: Involvement in ovulation. Endocrinology **116:**516–521, 1985.
33. Cooke PS, Kirby JD, Porcelli J. 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, 1993.
34. Kirby JD, Jetton AE, Cooke PS, Hess RA, Bunick D, Ackland JF, Turek FW, Schwartz NB. Developmental hormonal profiles accompanying the neonatal hypothyroidism-induced increase in adult testicular size and sperm production in the rat. Endocrinology **131:**559–565, 1992.
35. Mehendale RG, Bruot BC. Thyroid stimulating hormone inhibits rat granulosa cell steroidogenesis in primary culture. Endocrine **3:**215–220, 1995.
36. Maruo T, Katayama K, Barnea ER, Mochizuki M. A role for thyroid hormone in the induction of ovulation and corpus luteum function. Horm Res **37:**12–18, 1992.
37. Simorangkir DR, Wreford NG, De Kretser DM. Impaired germ cell development in the testes of immature rats with neonatal hypothyroidism. J Androl **18:**186–193, 1997.
38. Van Haaster LH, De Jong FH, Docter R, De Rooij DG. The effect of hypothyroidism on Sertoli cell proliferation and differentiation and hormone levels during testicular development in the rat. Endocrinology **131:**1574–1576, 1992.
39. Cooke PS, Zhao YD, Bunick D. Triiodothyronine inhibits proliferation and stimulates differentiation of cultured neonatal Sertoli cells: Possible mechanism for increased adult testis weight and sperm production induced by neonatal goitrogen treatment. Biol Reprod **51:**1000–1005, 1994.
40. Anasti JN, Flack MR, Froehlich J, Nelson LM, Nisula BC. A potential novel mechanism for precocious puberty in juvenile hypothyroidism. J Clin Endocrinol Metab **80:**276–279, 1995.
41. Hidaka A, Minegishi T, Kohn LD. Thyrotropin, like luteinizing hormone (LH) and chorionic gonadotropin (CG), increases cAMP and inositol phosphate levels in cells with recombinant human LH/CG receptor. Biochem Biophys Res Commun **196:**187–195, 1993.
42. Sanyal MK, Taymor ML, Berger MJ. Cytological features of oocytes in the adult human ovary. Fertil Steril **27:**501–510, 1976.

43. Uilenbroek JT. Hormone concentrations and ovulatory response in rats treated with antiprogestagens. J Endocrinol **129:**423–429, 1991.
44. Hubbard GM, Erickson GF. Luteinizing hormone–independent luteinization and ovulation in the hypophysectomized rat: A possible role of the oocyte. Biol Reprod **39:**183–194, 1988.
45. Risma KA, Hirshfield AN, Nilson JH. Elevated luteinizing hormone in prepubertal transgenic mice causes hyperandrogenemia, precocious puberty, and substantial ovarian pathology. Endocrinology **138:**3540–3547, 1997.
46. Mossman HW, Duke KL. Comparative Morphology of the Vertebrate Ovary. Madison, WI: The University of Wisconsin Press, 1973.
47. Mandl AM. Factors influencing ovarian sensitivity to gonadotropins. J Endocrinol **15:**448–457, 1957.
48. Tyndale HH, Levin L. Ovarian weight responses to menopause urine injections in normal hypophysectomized and hypophysectomized thyroxine–treated immature rats. Am J Physiol **120:**486–493, 1937.
49. Fitko R, Szlezyngier B. Role of thyroid hormone in controlling the concentration of luteinizing hormone/human chorionic gonadotropin receptors in rat ovaries. Eur J Endocrinol **130:**378–380, 1994.
50. Copmann TL, Adams WC. Ovarian gonadotropin receptors during experimental ovarian cyst formation in the rat. Biol Reprod **25:**115–119, 1981.
51. Mehendale RG, Bruot BC. TSH stimulates *in vitro* progesterone secretion by luteal cells from euthyroid and hypothyroid hCG-treated rats. Biol Reprod **52:**111, 1995.
52. Risma KA, Clay CM, Nett TM, Wagner T, Yun J, Nilson JH. Targeted overexpression of luteinizing hormone in transgenic mice leads to infertility, polycystic ovaries, and ovarian tumors. Proc Natl Acad Sci USA **92:**1322–1326, 1995.
53. Kamat BR, Brown LF, Manseau EJ, Senger DR, Dvorak HF. Expression of vascular permeability factor/vascular endothelial growth factor by human granulosa and theca lutein cells. Am J Pathol **146:**157–165, 1995.
54. Senger DR, Galli SJ, Dvorak AM, Perruzzi CA, Harvey VS, Dvorak HF. Tumor cells secrete a vascular permeability factor that promotes accumulation of ascites fluid. Science **219:**983–985, 1983.
55. Leung DW, Cachianes G, Kuang W-J, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. Science **246:**1306–1309, 1989.
56. Shweiki D, Itin A, Soffer D, Keshet E. Vascular endothelial growth factor induced by hypoxia may mediate hypoxia-initiated angiogenesis. Nature **359:**843–845, 1992.
57. Leathem JH. Biochemistry of cystic ovaries. Ciba Found Colloquia on Endocrinology **12:**173–189, 1958.