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Effects of Dihydrotestosterone on Progesterone Secretion in Pseudopregnant Rats (42426)

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Abstract. To determine if the administration of dihydrotestosterone (DHT) suppresses serum progesterone concentrations in pseudopregnant rats and if so what role the decidua play in mediating this effect, two pellets (8 mg) of DHT were inserted under each ovarian bursa on Day 9 of pseudopregnancy in hysterectomized or intact rats with or without the decidua. The treatment induced within 24 hr a 50% decline in serum progesterone concentrations in decidua-bearing rats only; a further reduction was observed on Days 11 and 12. To further determine if the effect of DHT on serum progesterone concentrations is due to its effect on luteal luteinizing hormone (LH)-receptor content, rats were similarly treated on Day 9 and the capacity of corpora lutea to bind ^{125}I-labeled human chorionic gonadotropin (hCG) on Day 12 in all three groups of rats was examined. DHT treatment had no effect on luteal LH-receptor content in any group. These results suggest that DHT is luteolytic in pseudopregnant rats only in the presence of the decidua and it is speculated that this effect is mediated by suppressing the decidual luteotropin.

Dihydrotestosterone (DHT) has been shown to cause follicular atresia in female rats (1) and reversible infertility in male rats (2). Plasma concentrations of DHT are significantly higher in nonpregnant women with recurrent pregnancy disorders of unknown etiology than the levels found in nonpregnant normal women (3). Administration of DHT on the day of metestrus in mice results in a significant reduction in the number of females becoming pregnant and bearing normal fetuses (4). These are only a few of the numerous studies which substantiate the antifertility effects of DHT. Our recent study demonstrates that DHT levels in the ovaries increase significantly at the end of pregnancy in the rat concomitant with the cessation of corpus luteum function and that DHT treatment during the first half of pregnancy suppresses serum progesterone concentrations within 24 hr, resulting in luteolysis and abortion subsequently (5). Because progesterone levels in the serum declined before fetal death occurred and DHT induced abortion only in rats with ovaries present, it was concluded that DHT was not directly detrimental to the fetuses but acted either directly on the ovaries or indirectly via the pituitary to inhibit progesterone synthesis (5). However, the finding that DHT causes abortion only when administered between Days 8 and 12 of pregnancy remained unexplained. At this stage of pregnancy, secretion of progesterone by the corpus luteum is maintained, in fact, by the decidual tissue. Since the earliest sign of abortion is the disintegration of the decidual tissue, it was of interest to determine whether DHT-induced luteolysis were due to its detrimental effect on this tissue. For this purpose we used pseudopregnant rats with or without decidual tissue. In these rat models, prolactin or decidual luteotropin acts in concert with either luteinizing hormone (LH) (6, 7) or luteal estradiol (7–9) to sustain progesterone secretion. LH is responsible for the availability of sufficient concentrations of estradiol in the corpus luteum (10) whereas prolactin or decidual luteotropin maintains and enhances receptors for estradiol (11) and thus permits estradiol to stimulate progesterone synthesis (12). Although decidual luteotropin possesses several physiological and biochemical characteristics of prolactin, it differs structurally (13) and immunologically (8) from prolactin in the rat. Therefore, the objective of the present study is to determine if the administration of DHT suppresses serum progesterone concentrations

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in pseudopregnant rats and if so what role the decidua might play in mediating this effect.

Materials and Methods. *General.* Pseudopregnant rats of Sprague–Dawley strain were purchased from Holtzman Company (Madison, Wisc.). They were housed in a temperature-controlled room (24–26°C) with automatically controlled lighting (lights on 0300 to 1700 hr CST daily) and were given free access to Purina rat chow and water. Pseudopregnancy was induced in female rats by vasectomized males on the afternoon of proestrus at the Holtzman facilities, and the following day (estrus) was considered the first day of pseudopregnancy. Only those rats showing diestrus smears subsequently were used in the experiment. On Day 5 of pseudopregnancy, under ether anesthesia, rats had their uterus scratched to induce decidual tissue formation, or were hysterectomized. A clean but not aseptic technique was used during all surgical procedures.

Treatment. DHT (5α-androstan-17β-ol-3-one; Steraloids) pellets were prepared by tamping the crystals into the cylindrical end of a pellet maker designed to make a pellet of a given weight per unit of length (14). Each pellet weighed about 4 mg. Two pellets were implanted under each ovarian bursa on Day 9 of pseudopregnancy through a small incision after laparotomy. Ovarian bursas were slightly cut in control.

Bleeding. Jugular vein blood was obtained by tapping the jugular vein through the unbroken skin under ether anesthesia. Blood was allowed to clot and centrifuged at 4°C. Serum was stored frozen at −20°C.

Radioimmunoassay of progesterone. Serum concentrations of progesterone were measured after hexane extraction using a highly specific antiserum provided by Dr. G. D. Niswender (GDN-337) and used by us as reported earlier (15).

Radioreceptor assay for LH. Rats were killed on Day 12 of pseudopregnancy by an overdose of ether. Ovaries were removed. Corpora lutea were carefully dissected out from the ovaries, weighed on a torsion balance, and stored frozen at −20°C. LH-receptor content in luteal tissue was determined by measuring the specific binding of ^{125}I-labeled human chorionic gonadotropin (hCG) to luteal cell membranes as has been described previously (16, 17). A crude luteal tissue membrane fraction from each animal was obtained by homogenization of the corpora lutea in phosphate-buffered saline (PBS), centrifugation of the homogenate at 13,000*g* for 20 min, and resuspension of the resulting pellet in PBS. Aliquots of membrane fractions were incubated for 4 hr at 22°C under gentle shaking with ^{125}I-labeled (New England Nuclear) hCG alone (30,000 cpm; 40 cpm/pg) or 1000-fold excess of unlabeled hCG. Reactions were terminated by dilution with cold buffer (1 ml PBS at 0°C) followed by centrifugation at 13,000*g* for 15 min at 0°C. The pellets were washed twice. ^{125}I-radioactivity was measured in the pellets with an automatic γ-counter with a counter efficiency of 70%. Specific binding was calculated as the difference between binding in the presence (nonspecific) and absence (total) of an excess of unlabeled hormone. Specific binding was expressed as cpm bound per corpus lutem.

Statistics. The data were analyzed for differences between groups by one-way analysis of variance followed by the Neuman–Keuls test when differences were significant. A P value of <0.05 was considered significant.

Results. *Effect of DHT treatment on serum progesterone concentrations (Fig. 1).* Two DHT pellets (8 mg) inserted under each ovarian bursa on Day 9 pseudopregnancy induced within 24 hr a 50% decline in serum progesterone concentrations in decidua-bearing rats. The treatment further suppressed the progesterone concentrations drastically in these rats when compared to controls on Days 11 and 12. In addition, all the decidua-bearing rats in the treated group showed vaginal bleeding on Day 12 which was indicative of the collapse of the decidua. DHT treatment on Day 9 in ordinary or hysterectomized rats had no effect on serum progesterone concentration on subsequent days when measurements were made. It appears from these data that the decidua play a key role in suppressing serum progesterone concentrations due to DHT treatment.

Effect of DHT treatment on luteal LH-receptor content (Table I). Treatment with two DHT pellets on Day 9 of pseudopregnancy had no effect by Day 12 on the capacity of corpora lutea to bind ^{125}I-hCG in all three groups of pseudopregnant rats.

Discussion. The results of this study dem-

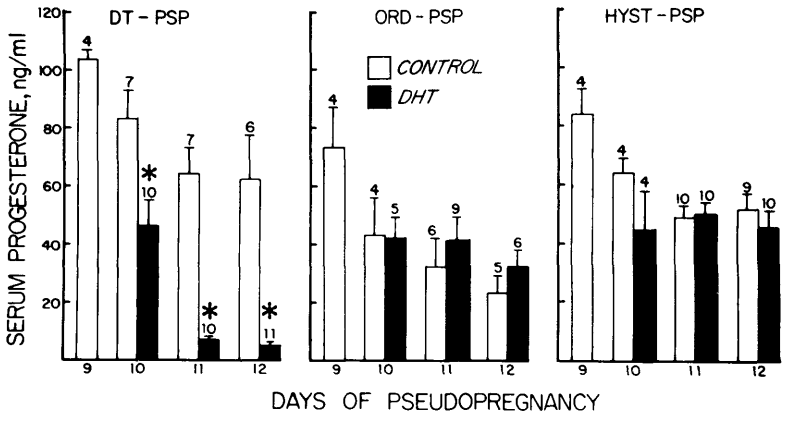

FIG. 1. Effect of dihydrotestosterone (DHT) on serum progesterone concentrations in pseudopregnant rats. Rats were bled from the jugular vein daily. Two DHT pellets (8 mg) were inserted under each ovarian bursa on Day 9 in treated group. Ovarian bursas were slightly cut in control. Each bar represents the mean ± SEM with the number of rats above each bar. $*P < 0.05$ with respect to its control each day. DT-PSP, decidua-bearing pseudopregnant; ORD-PSP, ordinary pseudopregnant; HYST-PSP, hysterectomized pseudopregnant.

onstrate that DHT, a metabolite of testosterone, possesses potent luteolytic activity when administered to pseudopregnant rats with decidualized uteri. In the absence of the decidual tissue, DHT had no detrimental effect on luteal secretion of progesterone. Since all three groups of pseudopregnant rats have intact pituitaries and ovaries, the DHT effect must be mediated by the decidual tissue.

In pseudopregnant rats, LH acts directly on corpora lutea to stimulate estradiol synthesis (18) which then acts in synergism with either prolactin or decidual luteotropin to sustain progesterone secretion (19, 20). DHT could have altered the sensitivity of corpora lutea to LH by lowering the luteal cell content of LH receptors. Such an action of DHT has been described in follicles (21). However, results of this investigation indicate that DHT does not affect binding sites for LH in the corpus luteum. Androgens have been shown in a number of systems to bind to the estrogen receptor (22). This action may prevent estrogen from binding to its receptor. Because corpora lutea are dependent on estradiol, such an action of DHT may have curtailed progesterone synthesis. However, it the DHT luteolytic action occurred via that mechanism, progesterone production would have decreased in each type of pseudopregnant rat studied and not only in the rats with decidual tissue. In the rat, decidual tissue of either pregnant or pseudopregnant rat produces a hormone, decidual luteotropin (13), which strongly influences luteal cell function (7–9, 13, 20, 23, 24). Twenty-four hours after implantation in pregnant rats or induction of decidual tissue in pseudopregnant rats, pituitary prolactin is no longer required because of the emergence of decidual luteotropin (7, 8). Decidual luteotropin binds to prolactin receptors in luteal cells and maintains progesterone production in the absence of prolactin (8, 13, 20, 25). Although, both prolactin and decidual luteotropin are present between Days 8 and 12 of pseudopregnancy,

TABLE I. LUTEAL CONTENT OF LH-RECEPTOR ON DAY 12 OF PSEUDOPREGNANCY

	Binding capacity to ^{125}I-HCG (cpm/corpus luteum)		
Treatment	ORD-PSP	DT-PSP	HYST-PSP
DHT[a]	2418 ± 482[b] (5)	1203 ± 277 (5)	5526 ± 1717 (3)
Control	1683 ± 426 (4)	1664 ± 408 (4)	3132 ± 1469 (3)

[a] Two DHT pellets (8 mg) were inserted under each ovarian bursa on Day 9 of pseudopregnancy.
[b] Values are means ± SEM (*n*).

only decidual luteotropin appears to be responsible for sustaining progesterone synthesis (20). Corpora lutea of rats with decidual tissue do not respond to prolactin. Administration of prolactin to hypophysectomized pseudopregnant rats sustains progesterone synthesis only in rats without decidual tissue (20). Therefore, it is possible that once under the influence of decidual luteotropin, luteal cells cease to respond to prolactin and involute if the decidual tissue is removed. Androgen receptors have been shown to be present in the rat uterus (26, 27), suggesting that exogenously administered DHT or any other androgen can reach the uterus and can act at the decidual tissue. Administration of DHT at moderate doses (500–1000 μg/day for 15 days) induces uterine and vaginal atrophy in mature female rats (28) while at higher doses (0.5–5.0 mg/day for 3 days) fails to stimulate endometrial mucosal cell hypertrophy in immature female rats (29). Hence, it is possible that the administration of DHT produced a collapse of the decidua due to its direct action on the decidua. Consequently, this may also lead to declining concentrations of decidual luteotropin. However, this action remains to be assessed in these rats. The luteolytic action of DHT in both pregnant and pseudopregnant rats may, therefore, be mediated by its detrimental effect on the decidual tissue.

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1. Nandedkar TD. Is dihydrotestosterone a cause of follicular atresia? Med Hypotheses **7**:801–808, 1981.
2. Lotz W, Krause R. Dihydrotestosterone causes reversible infertility in male rats. Fertil Steril **35**:691–695, 1981.
3. Tajic M, Gerencer M, Kastelan A. Plasma testosterone and dihydrotestosterone in normal and abnormal pregnancy. Experientia **37**:427–429, 1981.
4. Nandedkar TD, Munshi SL. Effect of dihydrotestosterone on follicular development, ovulation and reproductive capacity of mice. J Reprod Fertil **62**:21–24, 1981.
5. Sridaran R, Gibori G. Induction of luteolysis by dihydrotestosterone in the pregnant rat. Amer J Physiol **241** (Endocrinol Metab **4**):E444–E448, 1981.
6. Rothchild I, Pepe GJ, Morishige WK. Factors affecting the dependency on LH in the regulation of corpus luteum progesterone secretion in the rat. Endocrinology (Baltimore) **95**:280–288, 1974.
7. Basuray R, Gibori G. Luteotropic action of the decidual tissue in the pregnant rat. Biol Reprod **23**:507–512, 1980.
8. Gibori G, Rothchild I, Pepe GJ, Morishige WK, Lam P. Luteotropic action of the decidual tissue in the rat. Endocrinology (Baltimore) **95**:1113–1118, 1974.
9. Castracane VD, Rothchild I. Luteotropic action of decidual tissue in the rat: Comparison of jugular and uterine vein progesterone level and the effect of ligation of the utero-ovarian connections. Biol Reprod **15**:497–503, 1976.
10. Sridaran R, Gibori G. Intraovarian localization of LH/HCG stimulation of testosterone and estradiol synthesis in the pregnant rat. Endocrinology (Baltimore) **112**:1770–1776, 1983.
11. Basuray R, Jaffe RC, Gibori G. Role of decidual luteotropin and prolactin in the control of luteal cell receptors of estradiol. Biol Reprod **28**:551–556, 1983.
12. Gibori G, Keyes PL. Role of intraluteal estrogen with the regulation of the rat corpus luteum during pregnancy. Endocrinology (Baltimore) **102**:1176–1182, 1978.
13. Jayatilak PG, Glaser LA, Basuray R, Kelly PA, Gibori G. Identification and partial characterization of a prolactin-like hormone produced by rat decidual tissue. Proc Natl Acad Sci USA **82**:217–221, 1985.
14. Shelesnyak MS, Berenblum I. An implantor for forming and accurate placing of small pellets. Science **115**:378–379, 1952.
15. Gibori G, Antczak E, Rothchild I. The role of estrogen in the regulation of luteal progesterone secretion in the rat during the period after day 12 of pregnancy. Endocrinology (Baltimore) **100**:1483–1495, 1977.
16. Gibori G, Richards JS. Dissociation of two distinct luteotropic effects of prolactin: Regulation of luteinizing hormone-receptor content and progesterone secretion during pregnancy. Endocrinology (Baltimore) **102**:767–774, 1978.
17. Richards JS, Williams JJ. Luteal cell receptor content for prolactin (PRL) and luteinizing hormone (LH): Regulation by LH and PRL. Endocrinology (Baltimore) **99**:1571–1581, 1976.
18. Kalison B, Warshaw ML, Gibori G. Contrasting effects of prolactin on luteal and follicular steroidogenesis. J Endocrinol **104**:241–250, 1985.
19. Gibori G, Richards JS, Keyes PL. Synergistic effects of prolactin and estradiol in the luteotropic process in the pregnant rat: Regulation of estradiol receptor by prolactin. Biol Reprod **21**:419–423, 1979.
20. Gibori G, Basuray R, McReynolds B. Luteotropic role of the decidual tissue in the rat: Dependency on intraluteal estradiol. Endocrinology (Baltimore) **108**: 2060–2066, 1981.

21. Farookhi R. Effects of androgen on induction of gonadotropin receptors and gonadotropin-stimulated adenosine 3',5'-monophosphate production in rat ovarian granulosa cells. Endocrinology (Baltimore) **106**:1216–1223, 1980.
22. Ruh TS, Wassilak SG, Ruh MF. Androgen-induced nuclear accumulation of the estrogen receptor. Steroids **25**:257–273, 1975.
23. Gibori G, Kalison B, Basuray R, Rao MC, Hunzicker-Dunn M. Endocrine role of the decidual tissue: Decidual luteotropin regulation of luteal adenylyl cyclase activity, luteinizing hormone receptors, and steroidogenesis. Endocrinology (Baltimore) **115**:1157–1163, 1984.
24. Gibori G, Kalison B, Warshaw ML, Basuray R, Glaser LA. Differential action of decidual luteotropin on luteal and follicular production of testosterone and estradiol. Endocrinology (Baltimore) **116**:1784–1791, 1985.
25. Jayatilak PG, Glaser LA, Warshaw ML, Herz Z, Gruber JR, Gibori G. Relationship between luteinizing hormone and decidual luteotropin in the maintenance of luteal steroidogenesis. Biol Reprod **31**:556–564, 1984.
26. Giannopoulos G. Binding of testosterone to cytoplasmic components of the immature rat uterus. Biochem Biophys Res Commun **44**:943–951, 1971.
27. Giannopoulos G. Binding of testosterone to uterine components of the immature rat. J Biol Chem **248**: 1004–1010, 1973.
28. Beyer C, Cruz ML, Gay VL, Jaffee RB. Effects testosterone and dihydrotestosterone on FSH serum concentration and follicular growth in female rats. Endocrinology (Baltimore) **116**:1784–1791, 1985.
29. Armstrong DT, Moon YS, Leung PCK. Uterotropic effects of testosterone and 5α-dihydrotestosterone in intact and ovariectomized immature female rats. Biol Reprod **15**:107–114, 1976.

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