J. steroid Biochem. Vol. 36, Nos 1/2, pp. 175–179, 1990
Printed in Great Britain. All rights reserved

0022-4731/90 $3.00 + 0.00
Copyright © 1990 Pergamon Press plc

STEROID SULPHATASE ACTIVITY IN THE HUMAN OVARIAN CORPUS LUTEUM, STROMA, AND FOLLICLE: COMPARISON TO ACTIVITY IN OTHER TISSUES AND THE PLACENTA

RAY V. HANING JR,* RICHARD J. HACKETT, RENEE I. BOOTHROID and JACOB A. CANICK[1]

Departments of Obstetrics and Gynecology and [1]Pathology, Brown University and Women and Infants Hospital, Providence, RI 02905, U.S.A.

(*Received* 2 *September* 1989)

Summary—Steroid sulfatase activity was measured in 89 human samples, using dehydroepiandrosterone sulfate (DHEAS) as substrate. The lowest activity was that of follicular fluid which was significantly lower than that of other tissues tested (each $P < 0.01$). The steroid sulfatase activity of ovarian tissue taken collectively (corpus luteum, stroma, and follicles) was higher than that of other tissues taken collectively (abdominal skin, uterus, and fallopian tube) ($P < 0.001$), and the steroid sulfatase activity of either the follicle ($P < 0.01$) or the stroma ($P < 0.05$) was significantly greater than that of the corpus luteum. The geometric mean steroid sulfatase activity of the placenta was significantly higher than other tissues tested (each $P < 0.01$) and was 22-fold higher than that of the follicle, the tissue with the next highest activity. These data indicate that the human ovary (particularly the stroma and follicle) is capable of utilizing DHEAS, an adrenal product, as a substrate for production of other androgens such as dehydroepiandrosterone (DHEA), androstenedione, and testosterone.

INTRODUCTION

Dehydroepiandrosterone sulfate (DHEAS) is secreted primarily by the adrenal. It is present in human plasma at a concentration higher than that of any other steroid hormone, and plasma DHEAS gives rise to 32% of plasma dehydroepiandrosterone (DHEA) in normal women [1]. Although the secretion of DHEAS is known to be high during intrauterine existence, low during infancy and childhood, and to rise again shortly prior to puberty, neither the physiological role of plasma DHEAS in the child and adult nor the mechanisms of its regulation are completely understood. An 18 peptide fragment of proopiomelanocortin (POMC) has been reported to have adrenal androgen stimulating effects and may represent the long-sought cortical androgen stimulating hormone (CASH) [2].

In the adult, plasma DHEAS concentrations vary with age and sex [3]. A low plasma concentration of DHEAS [4] (or DHEA [5]) appears to be a statistical predictor of osteoporosis and of death due to cardiovascular disease and other causes [6]. Furthermore, plasma DHEAS is negatively correlated with the insulin response to glucose and positively correlated with red cell insulin binding [7]. Administration of exogenous DHEA to rats [8, 9], mice [10, 11], rabbits [12] and humans [13] has been shown to raise the plasma DHEAS concentration [13], to lower the serum low density lipoprotein concentration [13] and to have anti-obesity [8, 10, 11, 13], antidiabetic [11], and antiatherosclerotic [12] effects.

DHEAS serves as an important prehormone for estrogens in pregnancy [14] and may serve as a prehormone for androgens in the adult ovary [15]. In pregnancy, after secretion by the fetal or maternal adrenal, DHEAS circulates in the blood to the placenta where the steroid sulfatase enzyme plays a critical role in liberating DHEA prior to its metabolism to estradiol. X-linked sulfatase deficiency results in a marked decrease in estrogen production by the fetoplacental unit and the dermatologic condition, icthiosis, after birth [16]. Studies of sulfatase deficient placentae have shown that a single enzyme in the placenta is responsible for hydrolyzing the sulfate from such diverse molecules as DHEAS [17, 18]. 11-desoxycorticosterone-21-sulfate [17], pregnenolone sulfate [18] and estrone sulfate [18]. Steroid sulfatase is present within many tissues including the ovary [19, 20]. For DHEAS to serve as a prehormone in the ovary, either DHEA or androst-5-ene-3β, 17β-diol must be liberated from the sulfate prior to serving as a substrate for the 3β-hydroxysteroid dehydrogenase-isomerase enzyme.

The present study was designed to compare the activity of steroid sulfatase within three different ovarian structures (corpus luteum, stroma, and

*To whom correspondence should be addressed.

follicle) and to compare these activities with that of fallopian tube, uterus, abdominal skin and placenta.

EXPERIMENTAL

Human tissues

Human tissues were obtained after removal during surgery under a protocol approved by the Human Subjects Committee of Women and Infants' Hospital. The tissues were obtained shortly after removal and processed immediately. Representative samples of tissue were taken, and frozen at $-20°C$ until processed for the sulfatase assay. Studies performed on both fresh and frozen tissues from the same specimen showed no loss of activity due to freezing.

Sulfatase assay

The sulfatase assay was performed as previously described [21] with modifications. After thawing, all tissues were kept on ice until incubation. The tissue was minced in 5 vol 0.1 M potassium phosphate buffer pH 7.4 and homogenized using a Polytron (Brinkman, Westbury, N.Y.). Reaction tubes were prepared by adding 60 nmol of [7-^{3}H]DHEAS (sp. act. 0.01 Ci/mmol) in ethanol and taking them to dryness. This produced a final concentration of 150 μM substrate in the 0.4 ml reaction volume. Potassium phosphate buffer (0.3 ml) was added, and the reaction tubes were warmed to 37°C and kept at that temperature until the reaction was stopped. Both zero tubes and homogenate tubes were prepared in triplicate for each homogenate. At time zero 0.4 ml methanol was added to the zero tubes to stop the sulfatase reaction. The reaction tubes then received 0.1 ml tissue homogenate at 30-s intervals to allow time for subsequent processing. After 20 min, the reaction was stopped by adding 0.4 ml methanol. All reaction tubes were centrifuged at 1500 g for 5 min. A 50 μl sample of the supernatant from each tube was added to a silica G thin-layer plate (Analtech 0.25 mm) along with 0.5 μmol non-isotopic DHEA as carrier. The plates were run 3 times in 98% chloroform 2% methanol for 20 min and sprayed with primulin stain for localization of the DHEA band under long u.v. light. The DHEA containing band was scraped into a scintillation vial and counted in 5 ml Scint-A XF scintillation fluid (Packard, Downers Grove, Ill.). Non-enzymatic hydrolysis of the sulfate was corrected for by subtraction of the mean CPM in the "zero" tubes from that in the assay tubes. The amount of product formed was calculated from the specific activity of the DHEAS after correction for counting efficiency.

The protein content of the homogenate was determined using the Bio-Rad Protein Assay method of Bradford (Biorad, Richmond, Calif.). The sulfatase activity was expressed as pmol/min/mg protein since a saturating concentration of DHEAS was used and the velocity of the reaction was proportional to the enzyme concentration. The velocity was found to be zero-order at the concentration of DHEAS used.

Statistical analysis

Differences in the means between different tissues were compared using a one-way analysis of variance with the Minitab statistical program [22]. All data was coded $(X + 2)$ and log transformed prior to statistical analysis to correct for log-normal distribution of the data. The coding was necessary due to small negative values for sulfatase activity in some samples of skin and follicular fluid. Where multiple comparisons were made to a single control, the increased probability of finding a significant result was corrected for by using Dunnett's tables for multiple comparisons to a single control [23].

RESULTS

Sulfatase activity was determined in 89 samples of tissue. Placentae were obtained at term immediately after delivery. All other tissues studied were obtained from women with active ovaries who were not on oral contraceptives or other medications which could modify the activity of the hypothalamic–pituitary–ovarian axis and who were neither pregnant nor postmenopausal. Only 1 sample of a given tissue type was obtained from any one subject. The saturating substrate concentration was evaluated by plotting velocity versus substrate concentration for 5, 10, 20, 40, 75, 100, 150, and 200 μM DHEAS. The concentration of 150 μM DHEAS was determined to be optimum for the sulfatase assay. Representative examples of a plot of enzyme velocity versus substrate concentration and a Lineweaver–Burke plot for ovarian stromal tissue are shown in Fig. 1. There was no significant effect of the phase of the cycle on the sulfatase activity, so tissue specimens from the follicular and luteal phase were pooled for statistical analysis. The lowest sulfatase activity was found in follicular fluid with a geometric mean of -0.17 pmol/min/mg protein and 95% confidence limits of -0.17 to 0.78 pmol/min/mg protein after correction for coding. The negative value indicates that no detectable enzymatic sulfatase activity was present in follicular fluid. The value for follicular fluid sulfatase activity was significantly lower than that of abdominal skin or any other tissue studied ($P < 0.01$). The sulfatase activity of ovarian tissues taken collectively (corpus luteum, stroma, and follicles) was higher than that of other non-placental tissues taken collectively (abdominal skin, uterus, and fallopian tube) ($P < 0.001$) (Fig. 2). The sulfatase activity of either the follicle ($P < 0.01$) or the stroma ($P < 0.05$) was significantly greater than that of the corpus luteum. The geometric mean sulfatase activity of the placenta was 22-fold higher than that of the next highest activity, that of the follicle, or any other tissue ($P < 0.01$).

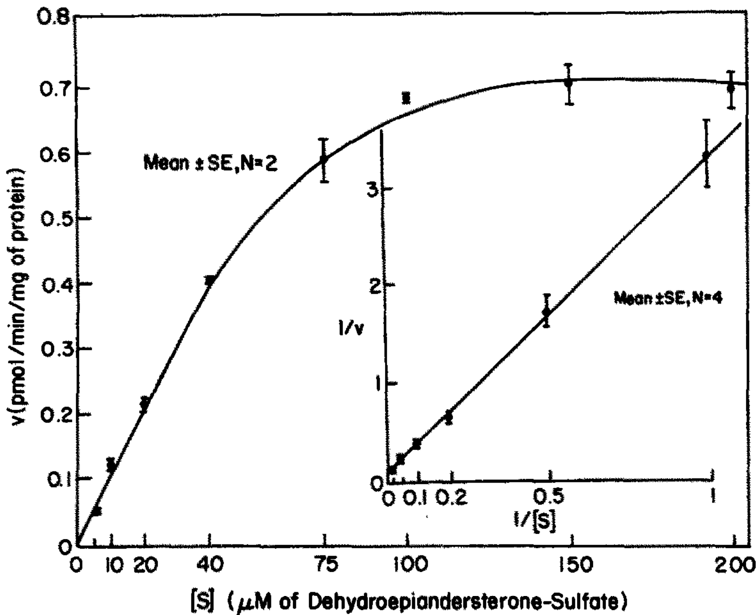

Fig. 1. Enzyme activity versus dehydroepiandrosterone sulfate (DHEAS) substrate concentration and a Lineweaver–Burke plot (inset) for representative samples of ovarian stroma.

DISCUSSION

A preliminary survey of the distribution of steroid sulfatases in human tissues was reported by Warren and French[20]. The sulfatase activity in the placenta was higher than in any other tissue examined. However, the wide range of values present within non-placental tissues required that a larger number of samples be tested to allow statistical analysis of differences in sulfatase activity between non-placental tissues. The high level of activity of sulfatase in human placenta reflects the critical role played by the sulfatase enzyme in the production of estrogen by the placenta [16].

Considerable evidence has accumulated for a role of steroid sulfates in metabolic pathways leading to secretion of testosterone in the testis [24]. Studies in the human have provided evidence that LH and hCG can stimulate sulfatase activity in the testis [25] and increase testicular secretion of steroid sulfates [26]. The high level of sulfatase enzyme activity in all ovarian structures compared to the tissues evaluated in the present study which do not secrete steroids suggests that steroid sulfates may also play a role as precursors for ovarian steroidogenesis, a possibility entertained by Warren and French[20]. The range of values in samples from different ovaries suggests both that the enzyme activity may vary with the

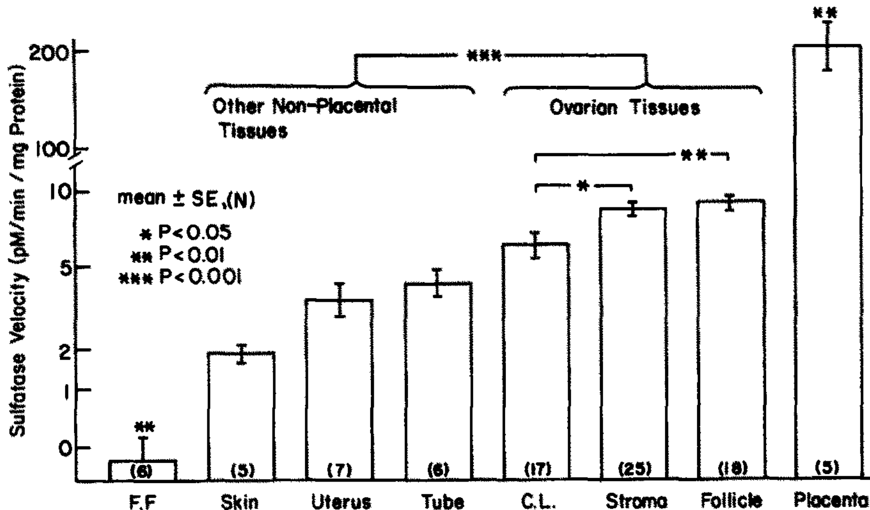

Fig. 2. Steroid sulfatase activity in follicular fluid (FF), abdominal skin, uterus, fallopian tube, corpus luteum (CL), ovarian stroma, ovarian follicles, and placenta. The scale is corrected for data coded as (X + 2) prior to log transformation, permitting zero and small negative values to appear.

steroidogenic activity of the ovarian structure sampled and that the sulfatase activity itself may be endocrinologically controlled. We found that both the follicle and the stroma had a higher geometric mean sulfatase activity than did the corpus luteum. The high level of activity in the stroma shows that DHEAS could be utilized by the stroma as a pre-hormone for androgen secretion, and the high level of steroid sulfatase in follicles suggests a similar possibility for DHEAS in the follicle.

These findings are consistent with our previous observation of a high correlation between the testosterone free index and the serum concentration of DHEAS [27] and also the statistical prediction of the testosterone free index by the serum DHEAS concentration and total ovarian volume in a multiple linear regression analysis [15]. The rapidly accumulating data on the pharmacological effects of administered DHEA in man and animals should be interpreted to include DHEAS as well since, (1) DHEAS has been shown to serve as a pre-hormone for 32% of plasma DHEA in normal women [1], (2) steroid sulfatase is capable of liberating DHEA from DHEAS within cells, (3) the concentration of serum DHEAS has been shown to rise when DHEA is administered, indicating that the DHEAS pool may be a major reservoir which maintains plasma and intracellular DHEA concentrations when DHEA is administered exogenously, and (4) DHEAS itself is secreted in large amounts by the adrenal, a unique occurrence among the steroid hormones which are, for the most part, secreted as non-conjugated steroids.

Evidence is building that adrenal androgen secretion may serve important physiological functions in the sexually maturing and sexually mature adults by: (1) adjusting metabolism to inhibit obesity and regulate the mass of adipose tissue relative to the mass of lean body tissues, (2) adjusting the insulin sensitivity of the tissues, (3) providing an anabolic steroid for muscle and bone in the absence of gonadal androgens such as androstenedione and testosterone.

The sulfatase enzyme may serve as a point of control for the intracellular pool of DHEA in that it would allow the cell to have access to the large pool of plasma DHEAS. The size of the plasma DHEAS pool and the concentration of plasma DHEAS could provide enough DHEA for all the androgens and estrogens synthesized by the ovary. However, parallel alternative pathways not involving sulfates make the steroid sulfate pathways less obvious. Studies of testicular function in sulfatase deficient males have shown that even though the testis in normal males may make extensive use of the steroid sulfates in synthesis of testosterone, the testis is able to compensate and produce normal quantities of testosterone using alternative (non-sulfate) pathways [28]. Likewise, administration of low doses of dexamethasone, sufficient to lower the plasma DHEAS concentration, to women lowered but did not block secretion of estradiol or androgens and did not inhibit ovulation [15].

Tracer studies in normal women showed that 32% of plasma DHEA arose from plasma DHEAS [1]. This determination does not measure DHEA which was derived from DHEAS and then metabolized further within the cell to other steroids. Presumably, each cell in the body has access to plasma DHEA, and those cells with the steroid sulfatase enzyme also have access to plasma DHEAS from which they can produce additional DHEA. Since plasma DHEAS circulates at a concentration approximately 1000-fold higher than that of plasma DHEA, the amount of intracellular DHEA derived from plasma DHEAS in cells with a very high steroid sulfatase activity theoretically could approach 1000-times that derived from plasma DHEA.

Androgens formed from plasma DHEAS in the ovarian stroma, theca or granulosa cells could gain access to the follicular fluid. Androgens not aromatized to estrogens within the follicle could be important locally in selection of the dominant follicle while those androgens re-entering the blood compartment could make a significant contribution to peripheral plasma DHEA, androstenedione, and testosterone. Although administration of DHEA to rats and mice tended to protect against tumor formation in many model systems [9], unexpected evidence of the possible importance of DHEA to the function of the ovary was provided by the observation that administration of DHEA to inbred strains of mice with an hereditary tendency towards development of ovarian granulosa cell tumors increased the rate of tumor formation. It also induced development of granulosa cell tumors in closely related strains which possessed some but not all of the genes necessary for spontaneous development of these tumors [29]. The human juvenile granulosa cell tumor has many similarities to the granulosa cell tumors seen in the mouse model [30]. High concentrations of plasma DHEAS of adrenal origin have also been reported in humans who subsequently developed gonadal tumors [31].

Acknowledgement—This work was supported in part by NIH Grant R01-HD-21280.

REFERENCES

1. Haning R. V. Jr, Chabot M., Flood C. A., Hackett R. and Longcope C.: Metabolic clearance rate of dehydroepiandrosterone sulfate (DS), its metabolism to dehydroepiandrosterone, androstenedione, testosterone, and dihydrotestosterone, and the effect of increased plasma DS concentration on MCR^{DS} in normal women. *J. Clin. Endocr. Metab.* **69** (1989) 1047–1052.
2. Parker L., Lifrak E., Shively J., Lee T., Kaplan B., Walker P., Calaycay J., Florsheim W. and Soon-Shiong P.: Human adrenal gland cortical androgen-stimulating hormone is identical with a portion of the joining peptide of pituitary pro-opiomelanocortin. *71st Annual Meeting of the Endocrine Society* (1989) Abstr. No. 299.
3. Orentreich N., Brind J. L., Rizer R. L. and Vogelman J. H.: Age changes and sex differences in serum dehydroepiandrosterone sulfate concentrations throughout adulthood. *J. Clin. Endocr. Metab.* **59** (1984) 551–555.

4. Deutsch S., Benjamin F., Tafreshi M., Kocheril G. and Frank A.: the correlation of serum estrogens and androgens with bone density in the late postmenopause. *Int. J. Gynaec. Obstet.* **25** (1987) 217–222.
5. Nordin B. E. C., Robertson A., Seamark R. F., Bridges A., Philcox J. C., Need A. G., Horowitz M., Morris H. A. and Deam S.: The relation between calcium absorption, serum dehydroepiandrosterone, and vertebral mineral density in postmenopausal women. *J. Clin. Endocr. Metab.* **60** (1985) 651–657.
6. Barrett-Connor E., Khaw K. and Yen S. S. C.: A prospective study of dehydroepiandrosterone sulfate, mortality, and cardiovascular disease. *New Engl. J. Med.* **315** (1986) 1519–1524.
7. Schriock E. D., Buffington C. K., Hubert G. D., Kurtz B. R., Kitabchi A. E., Buster J. E. and Givens R.: Divergent correlations of circulating dehydroepiandrosterone sulfate and testosterone with insulin levels and insulin receptor binding. *J. Clin. Endocr. Metab.* **66** (1988) 1329–1331.
8. Mohan P. F. and Cleary M. P.: Comparison of dehydroepiandrosterone and clofibric acid treatments in obese zucker rats. *J. Nutr.* **119** (1989) 496–501.
9. Schwartz A. G., Whitcomb J. M., Nyce J. W., Lewbart M. L. and Pashko L. L.: Dehydroepiandrosterone and structural analogs: a new class of cancer chemopreventitive agents. *Adv. Cancer Res.* **51** (1988) 391–424.
10. Yen T. T., Allan J. A., Pearson D. V. and Acton J. M: Prevention of obesity in A^{VY}/a mice by dehydroepiandrosterone. *Lipids* **12** (1977) 409–413.
11. Coleman D. L., Leiter E. H. and Schwizer R. W.: Therapeutic effects of dehydroepiandrosterone in diabetic mice. *Diabetes* **31** (1982) 830–833.
12. Gordon G. B., Bush D. E. and Weisman H. F.: Reduction of atherosclerosis by administration of dehydroepiandrosterone. A study in the hypercholesterolemic New Zealand white rabbit with aortic intimal injury. *J. Clin. Invest.* **82** (1988) 712–720.
13. Nestler J. E., Barlascini C. O., Clore J. N. and Blackard W. G.: Dehydroepiandrosterone reduces serum low density lipoprotein levels and body fat but does not alter insulin sensitivity in normal men. *J. Clin. Endocr. Metab.* **66** (1988) 57–61.
14. Gant N. F., Hutchinson H. T., Stiteri P. K. and MacDonald P. C.: Study of the metabolic clearance rate of dehydroisoandrosterone sulfate in pregnancy. *Am. J. Obstet. Gynec.* **111** (1971) 555–563.
15. Haning R. V. Jr, Austin G. W., Carlson I. H., Kuzma D. L. and Zwiebel W. J.: Role of dehydroepiandrosterone sulfate as a prehormone for ovarian steroidogenesis. *Obstet. Gynec.* **65** (1985) 199–205.
16. Hameister H., Wolff G., Lauritzen C. H., Lehmann W. O., Hauser A. and Ropers H. H.: Clinical and biochemical investigations on patients with partial deficiency of placental steroid sulfatase. *Hum. Genet.* **46** (1979) 199–207.
17. Egyed J. and Oakey R. E.: Hydrolysis of deoxycorticosterone-21-yl sulphate and dehydroepiandrosterone sulphate by microsomal preparations of human placentae: evidence for a common enzyme. *J. Endocr.* **106** (1985) 295–301.
18. France J. T., Seddon R. J. and Liggins G. C.: A study of a pregnancy with low estrogen production due to placental sulfatase deficiency. *J. Clin. Endocr. Metab.* **36** (1973) 1–9.
19. Birstein S. and Dorfman R. I.: Determination of mammalian steroid sulfatase with 7α-H^3-3β-hydroxy-androst-5-en-17-one sulfate. *J. Biol. Chem.* **238** (1963) 1656–1660.
20. Warren J. C. and French A. P.: Distribution of steroid sulfatase in human tissues. *J. Clin. Endocr. Metab.* **25** (1965) 278–282.
21. Platia M. P., Fencl M. deM., Elkind-Hirsch E., Canick J. A. and Tulchinsky D.: Estrone sulfatase activity in the human brain and estrone sulfate levels in the normal menstrual cycle. *J. Steroid Biochem.* **21** (1984) 237–241.
22. Ryan B. F., Joiner B. L. and Ryan T. A., Jr: *Minitab Handbook*, 2nd edn. Buxbury Press, Boston (1985) p. 206.
23. Dunnett C. W.: New tables for multiple comparisons with a control. *Biometrics* **20** (1964) 482–490.
24. Vihko R. and Ruokonen A.: Steroid sulphates in human adult testicular steroid synthesis. *J. Steroid Biochem.* **6** (1975) 353–356.
25. Payne A. H., Jaffe R. B. and Abell M. R.: Gonadal steroid sulfates and sulfatase III. Correlation of human testicular sulfatase, 3β-hydroxysteroid dehydrogenase-isomerase, histologic structure and serum testosterone. *J. Clin. Endocr. Metab.* **33** (1971) 582–591.
26. Laatikainen T., Laitinen E. A. and Vihko R.: Secretion of free and sulfate-conjugated neutral steroids by the human testis. Effect of administration of human chorionic gonadotropin. *J. Clin. Endocr. Metab.* **32** (1971) 59–64.
27. Haning R. V., Jr, Carlson I. H., Shapiro S. S. and Nolten W. E.: Testosterone free index correlates best with dehydroepiandrosterone sulfate. *Fert. Steril.* **36** (1981) 757–765.
28. Ruokonen A., Oikarinen A. and Vihko R.: Regulation of serum testosterone in men with steroid sulfatase deficiency: response to human chorionic gonadotropin. *J. Steroid Biochem.* **25** (1986) 113–119.
29. Beamer W. G., Tennent B. J., Shultz K. L., Nadeau J. H., Shultz L. D. and Skow L. C.: Gene for ovarian granulosa cell tumor susceptibility, Gct, in SWXJ recombinant inbred strains of mice revealed by dehydroepiandrosterone. *Cancer Res.* **48** (1988) 5092–5095.
30. Beamer W. G.: Gonadotropin, steroid, and thyroid hormone milieu of young SWR mice bearing spontaneous granulosa cell tumors. *J. Natn. Cancer Inst.* **77** (1986) 1117–1123.
31. Haning, R. V., Jr, Loughlin J. S. and Shapiro S. S.: Diagnosis and resection of an oral contraceptive suppressible Sertoli-Leydig cell tumor with preservation of fertility and a 7 year follow-up. *Obstet. Gynec.* **73** (1989) 901–905.