

Immunohistochemical Studies on Sex Steroid Hormones In Sex Cord-Stromal Tumors of the Ovary

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Summary: Estradiol, progesterone and testosterone were examined immunohistochemically in formalin fixed paraffin embedded tissue specimens of 15 sex cord-stromal tumors [6 granulosa cell tumors (GCT), 2 thecomas, 5 fibromas, 1 sclerosing stromal tumor and 1 Sertoli-Leydig cell tumor] of ovary using ABC method. In the granulosa cell tumor, estradiol, progesterone and testosterone were demonstrated in the granulosa cells. Clinical evidence of increased estradiol was found in 4/6 cases (66.7%) of GCT, being consistent with the steroid localization. In the thecomas, progesterone and testosterone were weakly positive in the luteinized theca cells, where immunoreactivity of estradiol was not observed. Progesterone and testosterone were weakly positive in the vacuolated and polygonal tumor cells but not the fibroblastic cells of sclerosing stromal tumor. Estradiol was weakly and testosterone were weakly or moderately positive in both the Sertoli and Leydig cells of Sertoli-Leydig cell tumor. The patient had clinical evidences of increased androgen production, manifested by virilization and increased serum hormone level of androgens. No hormone immunoreactivity was observed in any of the fibromas. From the results of this study it may be concluded that specific types of hormonally active cells in these ovarian tumors can produce estradiol, progesterone and testosterone, except fibroma.

The pathology of ovarian neoplasm is one of the most complicated subject in the field of gynecology. Because a greater variety of tumor arises from the ovary. The ovaries contain germ cells which are toti-potential and mesenchymal cells which are multi-potential, so when the ovary undergoes a neoplastic change almost any variety of tumor can occur (1). Of these, sex cord-stromal tumors are one of the most interesting tumors of the ovary those are well known for their capacity of producing steroid hormones (2). Histologically this group of tumors includes all those that contain granulosa cells, theca cells and their luteinized derivatives, Sertoli cells, Leydig cells and fibroblasts of gonadal stromal origin, singly or in different combinations and in varying stages of differentiations (3). During embryogenesis the gonad has the potential to develop into either

testis or ovary. In adult, undifferentiated cells of sex cord-stromal retain this bisexual potential and this may give rise to either granulosa cell or Sertoli cell tumor. Moreover, these tumors are often accompanied by a stromal reaction, in case of granulosa cell tumors this adds a thecomatous component to the tumor and in case of Sertoli cell tumors there is usually Leydig cell differentiation. In addition, stromal cell themselves can differentiate into either thecomas or Leydig cell tumor (4).

Most functioning ovarian tumors, which accounts for approximately 8% (4) of all ovarian tumors, are the sex cord-stromal tumors. These tumors have evoked considerable interest for their clinical manifestation and biochemical observations of high level steroid hormone production (5, 6, 7, 8). Various morphological studies, including ultra-structural studies (9) and histochemical study for identification of oxidative enzymatic system, are all indirect evidences of steroid hormones, but they fail to identify the exact steroid hormones produced by specific cells. In order to obtain a better understanding of sex steroid hormone production in sex cord-stromal tumor, it is necessary to localize the site of steroidogenesis. The present study was undertaken to clarify the localization of sex steroid hormones in sex cord-stromal tumors and to characterize the various types of hormones in these tumors.

Materials and Methods

Tissue

The tissue samples were taken from women who had undergone surgery for ovarian tumor at Nagasaki University Hospital between 1980-1991. This included 15 sex cord-stromal tumors of the ovary, where 6 were granulosa cell tumors, 2 thecomas, 5 fibromas, 1 sclerosing stromal tumor and 1 Sertoli-Leydig cell tumor. As control, 10 morphologically normal ovaries (5 corpus luteum and 5 pre-ovulatory follicles) were obtained from reproductive patients who underwent oophorectomy for various gynecological disorders. A representative section was chosen from each case and new sections, 4-5 μ thick were prepared

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from the corresponding formalin fixed, paraffin embedded block of tumor tissue specimen.

Antibodies

The specific antibodies used in this study were estradiol (mono-clonal antibody; Immunotech S. A., France, lot no. 09 09. 92), progesterone-11-BSA (Chemicon International INC., Temecula, CA, lot no. 081489CH2) and testosterone-2-BSA (Chemicon International INC., Temecula, CA, lot no 061190CHD2). The antibodies were diluted to 1:100 dilution with phosphate buffer saline (PBS) pH 7.5.

Immunostaining

Immunohistochemical staining was done by ABC complex method using Elite Vectastain Kit (Vector Laboratories, INC. Burlingame, CA) as described in the following.

The sections were dewaxed by xylene and hydrated through graded alcohol. Endogenous peroxidase activity was blocked by applying 0.3% hydrogen peroxide in methanol. After rinsing in 3 changes of PBS, the sections were covered with 1% normal serum to reduce the effects of non-specific tissue binding of immunoglobulin. After tipping, the sections were incubated with primary antibody for 24 hours at 4 °C. After rinsing in 3 changes of PBS the sections were covered by biotinylated anti-rat (for estradiol) and anti-mouse (for progesterone and testosterone) Ig G. Sections were rinsed in PBS and incubated with avidin biotin complex. After rinsing in PBS, the slides of peroxidase activity were visualized by incubating the sections with enzyme substrate diaminobenzidine (DAB). The slides were rinsed in running tap water, counterstained with Mayer's hematoxylin, and dehydrated and mounted. Positive controls included sections of normal ovary containing

pre-ovulatory follicles, corpus luteum and normal testis. A negative control was run parallel to each test section by omitting the primary antibody.

Assesment of immunoreaction

The following gradings of immunolocalization were adopted: More than 50% of tumor cells having immunopositivity, (+++); 25 to 50% of tumor cells having immunopositivity, (++); less than 25% of tumor cells having immunopositivity, (+); no tumor cells having immunopositivity, (-). The staining intensity of immunoreaction were denoted as negative, weak, moderate and strong, as compared to controls.

Results

Clinical data

The ages of the 15 women with sex cord-stromal tumors of ovary, ranged from 20 to 76 years with a mean of 56 years. And 73.4% of these women were post-menopausal. The obstetric history was, 3 nulliparous and the rest 12 were parous, where the parity ranged from 1 to 9 (Table 1). None of the women was pregnant at the time of diagnosis. The most common presenting symptom was an abdominal mass, occurring in 5 patients; 4 of the patient had abdominal pain; atypical genital bleeding occurring in 3 patients were post- menopausal (Table 2). Only 1 patient had clinical evidences of hirsutism.

The endocrinological variables were investigated in 9

Table 1. Sex cord-stromal tumors: clinical data and endometrial pathology

Case no.	Histological diagnosis	Age	Menopause	G/P	Tumor site	Operation	Endometrial pathology
1	GCT	41	-	1/1	L	LSO	No sample
2	GCT	58	+	4/3	R	RSO	No sample
3	GCT	45	-	7/3	R	TAH+BSO	Cystic hyperplasia
4	GCT	75	+	9/9	L	TAH+BSO	Cystic hyperplasia
5	GCT	65	+	0/0	L	TAH+BSO	Atrophic
6	GCT	74	+	6/5	L	TAH+LSO	Atrophic
7	Thecoma	20	-	0/0	L	LO	No sample
8	Thecoma	63	+	6/5	L	TAH+BSO	Cystic hyperplasia
9	Fibroma	76	+	6/6	L	TAH+BSO	Adenomatous hyperplasia
10	Fibroma	59	+	9/9	L	TAH+BSO	Cystic hyperplasia
11	Fibroma	78	+	2/2	R	TAH+BSO	Atrophic
12	Fibroma	45	+	2/1	L	TAH+BSO	Atrophic
13	Fibroma	56	+	4/3	R	TAH+BSO	Cystic hyperplasia
14	Sclerosing stromal tumor	27	-	1/1	L	LO	No sample
15	Sertoli-Leydig cell tumor	58	+	0/0	R	TAH+BSO	Atrophic

GCT = Granulosa cell tumor, G = gravidity, P = parity, L = left, R = right, LSO = Left salpingo-oophorectomy, RSO = Right salpingo-oophorectomy, TAH = Total abdominal hysterectomy, BSO = Bilateral salpingo-oophorectomy, LO = Left oophorectomy

Table 2. Symptoms in sex cord-stromal tumors of ovary

Symptoms	Abdominal mass	Abdominal pain	Abdominal fullness	Atypical genital bleeding	Hirsutism	Others
No. of cases	5 (33.4)	4 (26.7)	3 (20.0)	3* (20.0)	1 (6.7)	1 (6.7)

() represents % * Histologically the patients had granulosa cell tumor (GCT)

patients with sex cord-stromal tumors. The most striking finding was an elevated androgen level in the hirsute patient with a Sertoli-Leydig cell tumor. The serum levels of estradiol, progesterone and testosterone were significantly high, pre-operatively in 77.8% (7/9), 22.3% (2/9) and 44.5% (4/9) of the patients respectively (Table 3).

Table 3. Serum hormone concentration in sex cord-stromal tumors of ovary

Histology	Hormone	Estradiol pg/ml	Pro- gesterone ng/ml	Andro- stenedione ng/ml	Testo- sterone ng/dl
Granulosa cell tumor		517.0	3.7	8.5	4.5
Granulosa cell tumor		240.0	0.4	0.8	22.9
Granulosa cell tumor		61.2	0.9	3.3	0.1
Granulosa cell tumor		89.0	2.66	5.95	170.0
Thecoma		90.5	1.0	1.5	36.7
Thecoma		28.0	0.6	0.5	0.9
Fibroma		81.9	1.4	0.8	54.2
Fibroma		55.9	0.8	3.0	1.0
Sertoli-Leydig cell tumor		88.0	5.0	4.66	945.0

Endometrial pathology was detected in 11 patients, whose endometrium were available for histological examination. The endometrium was suspected for estrogenic stimulation, as manifestation of cystic hyperplasia (8) were found in 5/11 (45.5%) and 1/11 (9.0%) patient had an adenomatous hyperplasia at the time of surgery. The other 5/11 (45.5%) of the patients had an atrophic endometrium (Table 1). The serum levels of estradiol was increased in 4/6 cases with cystic and adenomatous hyperplasia.

Steroid immunoreactivity

The results of immunoreactivity of the estradiol, progesterone and testosterone are shown in table 4, 5 and 6. The localization of these steroids are summarized in Table 7.

Control: In normal ovaries, immunoreactivity of estradiol was localized in the granulosa cells of the pre-ovulatory follicles and in luteinized granulosa cells, while testosterone was detected in the theca interna and theca lutein cells. Immunoreactivity of progesterone was found in the theca interna of ovarian follicles and in luteinized derivatives of both granulosa and theca cells of corpus luteum. No significant reactivity was observed in other stromal cells or surface epithelium.

Granulosa cell tumor: Histologically the 6 GCT were diagnosed as adult type (10) where the granulosa cells were less pleomorphic, number of mitoses were few and the

Table 4. Immunohistochemical reactivity of estradiol in sex cord-stromal tumors of ovary

Histology	Neg- ative	Positive			Total
		Weak	Moderate	Strong	
Granulosa cell tumor (granulosa cell)	0	1	3	2	6
Thecoma (theca cell)	2	-	-	-	2
Fibroma (spindle cell)	5	-	-	-	5
Sclerosing stromal tumor (vacuolated cell)	1	-	-	-	1
Sertoli-Leydig cell tumor (Sertoli and Leydig cell)	-	1	-	-	1

Table 5. Immunohistochemical reactivity of progesterone in sex cord-stromal tumors of ovary

Histology	Neg- ative	Positive			Total
		Weak	Moderate	Strong	
Granulosa cell tumor (granulosa cell)	2	2	2	-	6
Thecoma (theca cell)	1	1	-	-	2
Fibroma (spindle cell)	5	-	-	-	5
Sclerosing stromal tumor (vacuolated cell)	-	1	-	-	1
Sertoli-Leydig cell tumor (Sertoli and Leydig cell)	-	1	-	-	1

Table 6. Immunohistochemical reactivity of testosterone in sex cord-stromal tumors of ovary

Histology	Neg- ative	Positive			Total
		Weak	Moderate	Strong	
Granulosa cell tumor	2	2	2	-	6
Thecoma	1	1	-	-	2
Fibroma	5	-	-	-	5
Sclerosing stromal tumor	-	1	-	-	1
Sertoli-Leydig cell tumor (Sertoli cell)	-	-	1	-	1
(Leydig cell)	-	1	-	-	

nuclei with grooving were present. The most common pattern was a follicular arrangement encountered in 4 instances. In this group 3 of the neoplasms were composed of diffusely arranged granulosa cells which in areas formed

Table 7. Sex cord-stromal tumors of ovary: cellular immunolocalization of estradiol, progesterone and testosterone.

Case no.	Histological diagnosis	Tumor cell type	Estradiol	Progesterone	Testosterone
1	GCT	granulosa cell	+	++	++
		stromal cell	-	-	-
2	GCT	granulosa cell	++	+	+
		stromal cell	-	-	-
3	GCT	granulosa cell	+++	++	++
		stromal cell	-	-	-
4	GCT	granulosa cell	++	+	-
		stromal cell	-	-	-
5	GCT	granulosa cell	++	-	+
		stromal cell	-	-	-
6	GCT	granulosa cell	+++	-	-
		stromal cell	-	-	-
7	Thecoma	theca cell	-	+	-
		stromal cell	-	-	-
8	Thecoma	theca cell	-	-	+
		stromal cell	-	-	-
9	Fibroma	spindle cell	-	-	-
10	Fibroma	spindle cell	-	-	-
11	Fibroma	spindle cell	-	-	-
12	Fibroma	spindle cell	-	-	-
13	Fibroma	spindle cell	-	-	-
14	Sclerosing stromal tumor	vacuolated cell	-	+	+
		fibroblastic cell	-	-	-
15	Sertoli-Leydig cell tumor	Sertoli cell	+	-	+
		Leydig cell	+	+	+

GCT = Granulosa cell tumor, * luteinized type

lacunae i. e., simulating Call-Exner bodies of developing graafian follicles and 1 of the neoplasm was of macrofollicular type, characterized by cysts lined by well differentiated granulosa cells. In 1 tumor the pattern was of mixed trabecular and diffuse pattern in which the granulosa cells were arranged in bands separated by a fibromatous stroma. In other 2 cases the tumors were diffuse pattern, characterized by nest of granulosa cells where the nuclei were typically uniform, pale and often grooved. Immunoreactivity of estradiol was observed in granulosa cells in all the 6 cases (Fig. 1). The staining in the cytoplasm of the granulosa cells were strongly positive in 2/6, where clinically both had increased serum level of estradiol and 1 of the patient had cystic hyperplasia of the endometrium. Moderately positive staining in 3/6 and weakly positive staining in 1/6 were observed in these tumors, where 2 of them had clinical evidence of atypical genital bleeding with increased serum level of estradiol. The immunolocalization of estradiol was pre-dominantly positive in the histologic pattern of follicular type of GCT. The reaction for testosterone present in granulosa cells were almost invariably moderately (2/6)(Fig. 2) or weakly (2/6) positive, where clinically serum testosterone was increased in only 1 of the cases with an atrophic endometrium. The immunopositivity of testosterone was dominant in the histologic pattern other than the microfollicular type. The immunoreaction for progesterone revealed 2/6 of moderate (Fig. 3) and 2/6 of weak staining in the granulosa cells. The immunopositivity

of progesterone was predominant in the histologic pattern associated with diffuse type. The stromal cell surrounding the granulosa cells was consistently negative for all steroid hormones.

Thecoma: The 2 cases of thecomas were both luteinized type. Immunoreactivity of progesterone in 1/2 and testosterone in 1/2 of weak staining was present in these cases of thecomas. Both the steroids were localized in the luteinized theca cells, which manifested as clusters of large poly-

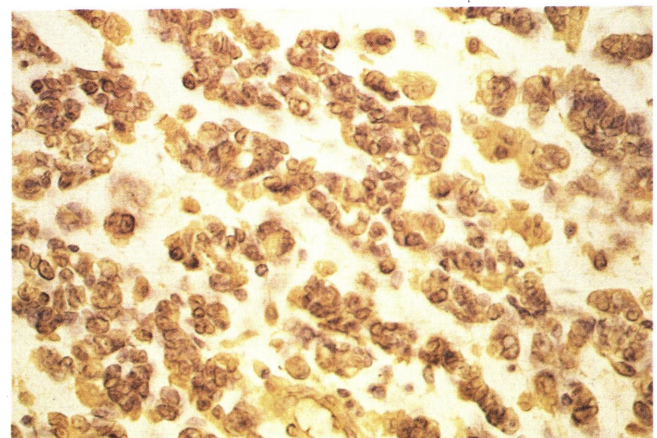


Fig. 1. Immunohistochemical reactivity of estradiol in granulosa cell tumor of ovary. Strongly positive reaction is noted in the cytoplasm of granulosa cells (x400).

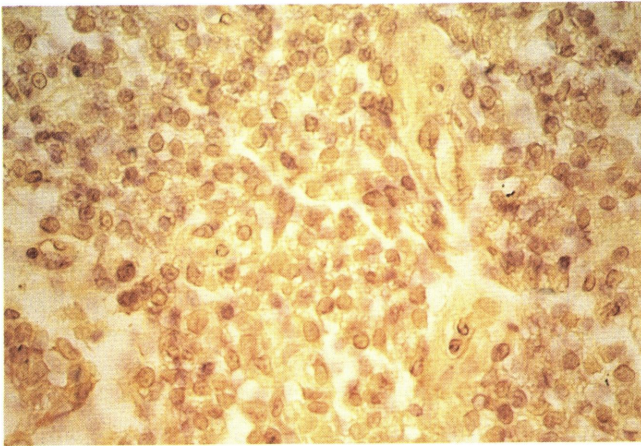


Fig. 2. Immunohistochemical reactivity of testosterone in granulosa cell tumor of ovary. Moderately positive reaction is noted in the cytoplasm of granulosa cells (x400).

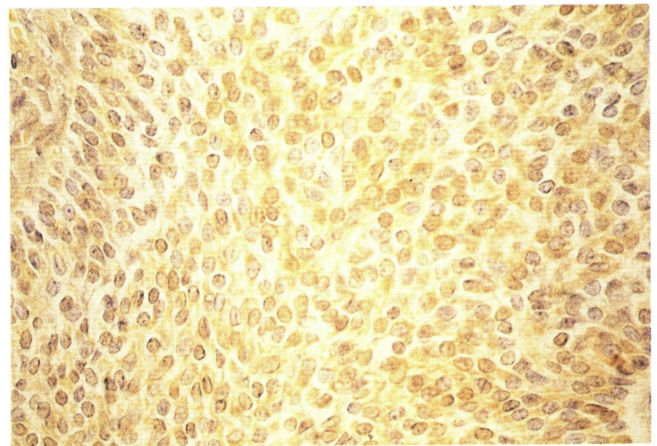


Fig. 3. Immunohistochemical reactivity of progesterone in granulosa cell tumor of ovary. Weak intracytoplasmic localization of progesterone is noted in the granulosa cells (x400).

hedral cells with abundant cytoplasm (Fig. 4). The other stromal cells forming the intersecting fascicle were negative for all the hormones. No significant immunoreactivity of estradiol was found in either cases of thecoma.

Fibroma: Five cases of fibroma were examined for the immunoreactivity of estradiol, progesterone and testosterone. The test revealed negative staining for all these three steroids in the fibroblastic cells in all the five cases.

Sclerosing stromal tumor: The tumor cells were an admixture of vacuolated, polygonal cells and fibroblastic cells which were separated by a hypocellular and edematous connective tissue areas. Immunoreactivity of progesterone and testosterone was weakly positive in the vacuolated cytoplasmic polygonal tumor cells (Fig. 5). No significant reactivity of estradiol was found in these polygonal tumor cells. No immunoreactivity of estradiol, progesterone and testosterone was observed in any fibroblastic cells.

Sertoli-Leydig cell tumor: Histologically, the case was a well differentiated type, which composed of hollow tubules lined by Sertoli cells and accompanied by clusters of Leydig cells. These Sertoli cells were columnar with basally placed nuclei and the cytoplasm had areas of vacuolation. An oil red O staining revealed lipid deposition in the vacuolated cells. The Leydig cells were round to polygonal with rounded nuclei with prominent nucleoli. Crystals of Reinke were detected both by light and electron microscopy in this case. Testosterone was moderately positive in the cytoplasm of the Sertoli cell and weakly positive in Leydig cells of the tumor. And greater number of Sertoli cells were positive for testosterone than Leydig cells (Fig. 6). Immunoreactivity for estradiol was clearly positive in some of the Leydig cells. A weak positive immunoreactivity of estradiol was observed in some of the columnar cells lining tubules. There was a correlation between clinical evidence of androgen excess, serum testosterone levels and cellular localization of testosterone.

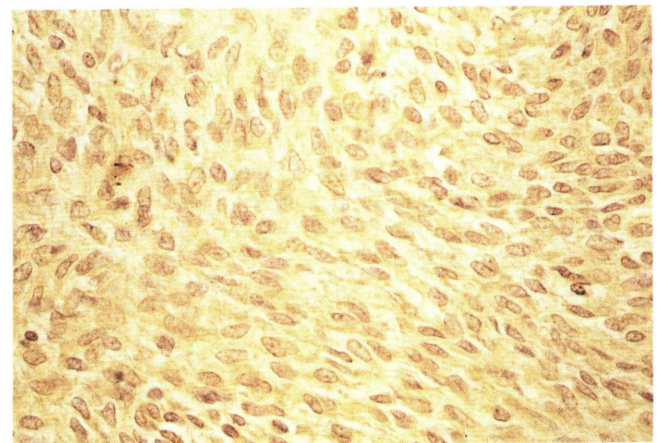


Fig. 4. Immunoreactivity of progesterone in luteinized thecoma. Weak immunoreactivity is observed in luteinized cells (x400).

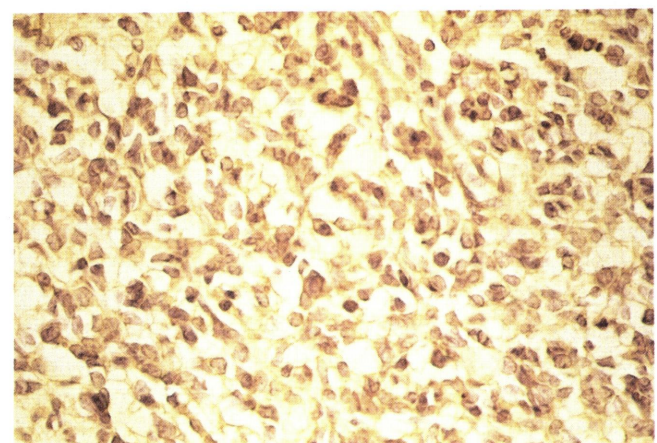


Fig. 5. Immunoreactivity of testosterone in sclerosing stromal tumor (x400). Weakly positive reaction is noted in the vacuolated cells.

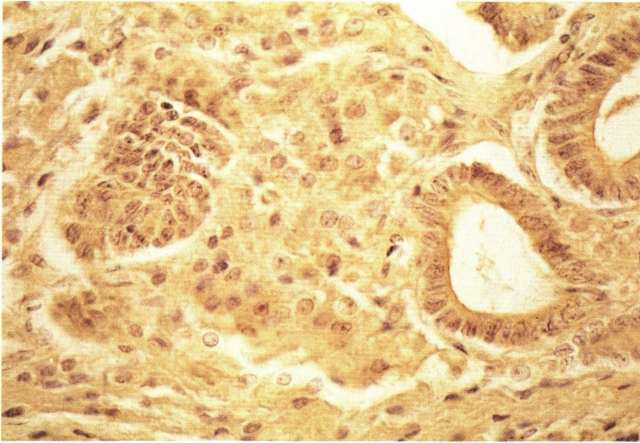


Fig. 6. Moderate intracytoplasmic localization of testosterone in Sertoli cells within a tubule and weakly positive reaction in Leydig cells. The tumor is a well differentiated Sertoli-Leydig cell tumor (x400).

Discussion

Hormone production by the ovary usually has been related to specific cell types. Previously, estrogen synthesis was attributed to theca cells and progesterone to luteinized granulosa cells of the corpus luteum. And non-luteinized granulosa cells of developing follicles and granulosa cell tumors were not considered to contribute in hormone synthesis (11). But, recently there are reports of in-vitro culture and auto-radiographic studies evidencing that granulosa cells of pre-ovulatory follicles and granulosa cell tumors do play a role in hormone synthesis (12, 13).

Almost 80% sex cord-stromal tumors of the ovary belong to the granulosa-theca group (14). Granulosa cell tumors have been known for many years to be associated with estrogen secretion. The source of the elevated serum and urinary estrogen level and tumor tissue estrogen in patients with granulosa cell tumors is attributed to the theca cells (15), the view based on the findings of immunohistochemical studies (16, 17), those demonstrates various types of lipid content or oxidative enzyme activity, are usually positive in the theca cells and negative or weakly positive in granulosa cells of a tumor containing both cell types. These findings and ultrastructural observations (18) have led to the conclusion that theca cell component of granulosa cell tumor is responsible for estrogen secretion. However, in more recent histochemical study, the graafian follicle granulosa cells and luteinized granulosa cells have been shown to have greater enzyme activity and other studies demonstrated that antral follicle granulosa cell contains various enzymes, actively involved in estrogen synthesis (19). But no tentative conclusion can be made based on these histochemical investigations, because the staining merely locate certain lipid content or certain enzymes involved in the steroidogenesis, but they fail to

identify the particular steroids themselves.

The ABC immunoperoxidase method demonstrating specific-cellular antigens has been applied in pathological diagnosis and it may be regarded as a very sensitive and specific immunologic staining method. This immunoperoxidase staining method might also stain hormones that is being stored (but not necessarily synthesized) in the tumor cells.

The data in this report is consistent with the view that the granulosa tumor cells are capable of synthesizing various sex steroid hormones. Assessing from the immunohistochemical reaction, estradiol was localized in the granulosa cells in the 6/6 cases. There are several possible explanation of the positive immunoreactivity to interpret the cytoplasmic localization of estradiol, as compatible with estrogen biosynthesis by the tumor cells (20, 21). Although in the present study a positive reaction does not necessarily imply steroid synthesis, since the immunohistochemical technique cannot distinguish biosynthesis from intracellular storage or binding to specific hormone receptor in the cells. The results of this report should be considered in conjunction with recent in vitro steroid metabolic studies which have demonstrated that granulosa cells when grown in culture containing FSH produce estradiol (22). And the granulosa cells of pre-ovulatory follicle not only produce more estradiol but also produce more cAMP and both are key regulators of granulosa cell differentiation (12). Furthermore, ultrastructurally, a small population of granulosa tumor cells of granulosa cell tumor contained mitochondria with tubular cristae and well developed smooth endoplasmic reticulum, organelles characteristics of steroid-secreting cells (13) and immunohistochemically estradiol was localized in those cell populate. In more recent reports, immunohistochemically P450 aromatase, the key regulatory enzyme for catalyzing the final step of androgen to estradiol, though of weak intensity was detected in one of the granulosa cell tumor case (23). Based on these facts and combining the endocrine and endometrial finding of this study, it is reasonable to assume that neoplastic granulosa cells have potential for estradiol secretion. The positive reaction of testosterone in granulosa cell in the present report is not unexpected since granulosa cells tumors have been reported to synthesize androgens, even with the evidence of virilization (24). More-over a significant 17α hydroxylase cytochrome P-450 activity, the key regulatory enzyme for conversion of progesterone to androgen has also been reported in human granulosa cell (25). Thus the localization of testosterone in the patients with granulosa cell tumor in the present report, who also had evidences of increased androgen production is in accord with this tumor.

Thecomas and fibromas are rarely found in surgical pathology specimen. Histologically, thecomas are classified into the typical form and luteinized thecoma (15). Luteinized thecomas contain cells with morphologic features of

steroid hormone secreting cells (5). In the present study, progesterone and testosterone were positive in luteinized cells in the thecomas, suggesting that these cell are associated with the production of progesterone and testosterone. The immunoreactivity of progesterone and testosterone is not unlikely as because the regulatory enzymes necessary for the final step of catalyzing the pregnenolone to progesterone and to androgen has already been immunohistochemically demonstrated in theca interna of well developed follicle, luteinized theca cells of corpus luteum and thecomas (23, 26). Estrogenic manifestations has also been reported in 23 of 46 cases of luteinized thecoma (8) but not in the present study, though one of the patients had increased serum estradiol and cystic hyperplasia of the endometrium. The absence of estradiol immunoreactivity in this case suggests that most of the estrogen produced may be due to peripheral or nonneoplastic ovarian conversion of androgen to estrogen.

According to the proposal of Young and Scully (5), thecoma should be diagnosed only by the evidence of steroid production. No immunoreactivity of estradiol, progesterone or testosterone was found in the spindle cell component of thecoma, in any fibroma or in normal ovarian stromal cells in this study. The fibromas are functionally inert. Though microscopically lipid content are occasionally demonstrated in fibromas, the immunoreactivity of cytochrome P450 and enymes for steroid synthesis were all negative (23).

Sclerosing stromal tumor was first reported by Chalvardjian and Scully (27) followed by a report of number of cases with possible steroid hormone production, including immunohistochemical localization of testosterone and androstenedione (28). In the present study a positive immunoreactivity of progesterone and testosterone was observed in vacuolated, polygonal tumor cells but not in fibroblastic cells. The results are compatible, as because the key regulatory enzymes (P-450 17 α and P-450 scc) necessary for the synthesis of these hormones has been detected in similar types of tumor cells of sclerosing stromal tumors (23).

Immunoreactivity of the estradiol and testosterone was observed in some of the Leydig cells and Sertoli cells lining tubules in the case of Sertoli-Leydig cell tumor, is consistent with standard morphologic observation of these cells as steroid producing cells. Ultrastructural studies have shown that both Sertoli and Leydig cells have lipid droplets, well developed smooth endoplasmic reticulum and elongated mitochondria with tubular cristae (14). According to in vitro studies and auto-radiographic analysis, Leydig cells produce both androgen (29) and estrogen (30). Again there are evidences in human and in animals that Sertoli cells produce both testosterone and estrogen (31, 32). The findings in this study are consistent with these observations since testosterone and estradiol were present in both the Sertoli and Leydig cells. And clinically this patient had

evidences of high levels of serum androgen too.

Hormone negative areas or cells of certain tumors were also detected, which may represent an inappropriate sampling or may in fact be composed of tumor cells with absent or minimal hormone synthesizing capacity which is therefore not detectable by the present method (13). Furthermore, as steroid are soluble in organic solvents, it is quite probable that a considerable amount of steroid was lost during the fixation and processing. Though immunoperoxidase method is nevertheless sufficiently sensitive to stain any remaining intracellular steroid (20). In summary this investigation has demonstrated immunohistochemical localization of estradiol, progesterone and testosterone in certain cells of various sex cord-stromal tumors. It appears that specific types of hormonally active cells in these ovarian neoplasm can produce a wide range of steroid hormones. The capacity of such diverse hormone synthesis can explain the observation of contradictive virilizing effects of certain granulosa cell tumor and estrogenic manifestation of certain Sertoli-Leydig cell tumor (32).

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