

Steroid Production in Different Parts of Malignant and Benign Ovarian Tumors *in Vitro*¹

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ABSTRACT

Pieces of ovaries and tumors from 45 patients (19 with malignant epithelial tumors, 14 with benign epithelial tumors, and 12 with normal postmenopausal ovaries) were incubated, and the release of steroid hormones from different parts of the tumors and from the contralateral ovaries was measured. Tumor tissue (mainly tumor cells with a small number of stromal cells), tumor base tissue (more stromal cells than tumor cells), and control ovaries were preincubated in oxygenated 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-minimum essential medium buffer at 37°C for 30 min followed by a 3-h incubation in fresh, oxygenated medium. Progesterone, androstenedione, testosterone, and estradiol were measured in the medium by radioimmunoassay at the end of the incubation period.

Malignant tumors released more progesterone and androstenedione than benign tumors or postmenopausal control ovaries. In contrast, benign tumors released more testosterone than malignant tumors or control ovaries. Release of estradiol was low and not significantly different among control ovaries and malignant and benign tumor tissue.

Different parts of the tumors differed in steroid hormone release. Tissue samples containing more tumor cells than stromal cells released more progesterone than those with predominantly stromal cells. Thus, malignant tumors had an active steroid secretion. Progesterone was the main steroid released.

INTRODUCTION

In a series of reports from this group and others it has been shown that women with ovarian epithelial carcinomas have elevated plasma levels of steroid hormones. The steroid concentration in blood parallels the tumor volume (1–8), increases with an increase in tumor size (1–3, 5), and increases prior to tumor recurrence (9). The concentration of progesterone at diagnosis is related to prognosis (10). Not only malignant but also benign ovarian tumors produce steroids (8, 11–13).

The production site is believed to be the ovary containing the tumor since a higher concentration is found in the ovarian vein draining the tumorous ovary as compared with the contralateral ovarian vein (7, 11, 14–15). Following oophrectomy plasma levels of androstenedione are decreased, suggesting that the origin of the steroid production is the tumorous ovary (13). There are reports showing that the remaining stroma of the ovary is activated in a postmenopausal ovary containing cancerous tissue, with signs of steroid production (16–17). Rome *et al.* (18) described characteristic changes of condensation and/or luteinization in tumors associated with high urinary excretion of estrogen, indicating that it was not the tumor cells but the activated remaining stroma that produced the steroid. Some authors suggest that the hyperplastic stroma of the tumorous ovary produces androgens which can be peripherally aromatized to estrone and estradiol (19, 20). Alternative pathways may contribute, such as conversion of adrenal

steroids. Elevated levels of dehydroepiandrosterone sulfate, an androgen of adrenal origin, was found in the serum of patients with “non-endocrine” ovarian carcinoma (21). Poels *et al.* (22) have characterized an ovarian carcinoma cell line which is capable of releasing estradiol into the culture medium; this indicates that hormone production by the tumor cells *per se* should be considered an additional possibility. These results are supported by Wimalasena *et al.* (23), who cultured cells from *in situ* epithelial ovarian carcinoma and demonstrated secretion of estradiol and progesterone.

In order to assess the production site of steroid hormones, tissues from different parts of malignant and benign tumors and from contralateral ovaries were studied *in vitro*.

MATERIALS AND METHODS

Patients. Forty-five women were recruited at the Departments of Obstetrics and Gynecology and Gynecological Oncology, University Hospital of Umeå (Umeå, Sweden), from February 1987 to October 1991. The mean age was 60 years (range, 28–84 years). Thirty-five women were postmenopausal. Nineteen had malignant or borderline epithelial tumors, and 14 had benign epithelial tumors. Twelve patients with normal postmenopausal ovaries constituted the control group. Histological examination revealed the following division into Federation Internationale des Gynecologues et Obstetristes types (24): serous carcinoma ($n = 7$); endometrioid carcinoma ($n = 5$); clear-cell carcinoma ($n = 1$); anaplastic carcinoma ($n = 1$); serous tumor of borderline malignancy ($n = 3$); mucinous tumor of borderline malignancy ($n = 2$); benign serous tumors ($n = 8$); and benign mucinous tumors ($n = 6$). Six of the carcinomas were poorly and eight moderately differentiated. Seven patients with carcinomas suffered from Federation Internationale des Gynecologues et Obstetristes stages III–IV disease (extension outside pelvis), and seven patients had stages I–II disease (pelvic extension) (24). Tissue from the contralateral ovary was incubated in 18 of 33 tumor cases. In 10 patients, six with malignant and four with benign tumors, the contralateral ovary contained only stromal cells. In the remaining cases the tumors were bilateral.

Operative Procedure. All patients were subjected to laparotomy due to pelvic masses. The abdominal wall and the peritoneum were opened, taking care to restrict leakage of blood into the peritoneal cavity. Ascitic fluid was collected and stored on ice while awaiting tumor surgery. In cases with no ascites the abdominal cavity was rinsed with 500–1000 ml physiological saline, and the fluid was aspirated and stored as above. Once the tumor had been removed, tissue samples were taken from two different parts of the tumor. One sample was taken from any part of typical viable tumorous appearance (“tumor”), and the other was taken from just beneath the oviduct (“tumor base”). The latter was chosen from an easily identified structure where tissue of remaining ovarian stromal origin usually could be found. In addition, one tissue sample was taken from the contralateral ovary. Each tissue piece was divided into two; one part was sent for histological examination, and the other was stored in a transport medium, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid-minimum essential medium buffer (pH 7.4) and cooled on ice until incubation.

Histological Examination. The relationship between tumor cells and stromal cells in each tissue sample was estimated using the method of point counting (25). A test grid containing 86 points was randomly laid on each microscopic image. Ten fields ($\times 120$ magnification) per image were counted manually. Tissue samples were mostly a mixture of tumor cells and histologically normal stromal cells. However, “tumor” tissue samples from carcinomas contained more tumor cells ($69.4 \pm 5.4\%$, mean \pm SEM) than stromal cells.

On the contrary, tissue samples from “tumor base” contained more stromal cells ($82.3 \pm 7.0\%$) than tumor cells. In tissue from tumors of borderline

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malignancy "tumor" samples contained 28.2% tumor cells ($\pm 12.5\%$), while "tumor base" tissue was made up of 9.8% ($\pm 7.9\%$) tumor cells. In the benign cases both "tumor" and "tumor base" samples contained mostly stromal cells and a small amount ($6.4 \pm 1.0\%$ and $4.4 \pm 1.2\%$, respectively) of epithelial cells outlining the cysts.

Incubation Procedure. Tissue from each station (tumor, tumor base, contralateral ovary, and control ovary) was kept on ice during dissection under the microscope to remove surrounding tissues and large blood vessels; then it was divided into four small pieces of about 10–30 mg each. After wet weight measurements, the pieces were put in air-tight glass vials with 1 ml of minimum essential medium buffered with 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Sigma, St. Louis, MO), with Earle's salt and L-glutamine (Gibco Laboratories, Grand Island, NY). The material was oxygenated with 100% O₂ for 1 min (26), preincubated in a gently gyrating water bath at 37°C for 30 min, put into another vial, oxygenated, and then incubated for 3 h at 37°C with the same amount of fresh medium (16). All incubations were performed in quadruplicate. After incubation the tissue was removed and the medium stored at -20°C until analysis. In order to investigate the effect of substrate on androstenedione and estradiol release, tissues from postmenopausal ovaries of five control patients were incubated in quadruplicate with the addition of progesterone 100 ng/ml (Sigma) or testosterone 100 ng/ml (Sigma).

Biochemical Assay. The progesterone, androstenedione, estradiol, and testosterone radioimmunoassays have been described earlier in detail (27, 28). Progesterone and estradiol were analyzed in medium from all 45 patients. Due to a shortage of medium androstenedione was analyzed in medium from 29 patients including all controls and testosterone was assayed in medium from 16 other patients and 6 controls. All samples from the same patient were assayed in the same run. The interassay coefficients of variation were 12%, 10%, 13%, and 10%, and the intraassay coefficients of variation were 8%, 8%, 10%, and 8% for progesterone, androstenedione, estradiol, and testosterone assays, respectively.

Statistics. The mean of each set of quadruplets was calculated and used for further statistical analysis. Two-way analysis of variance was used when several locations were simultaneously analyzed. If the first analysis of variance was significant, the differences between steroid levels in various tissue incubations were tested ad hoc by using the LSDT³ at 0.05, 0.03, and 0.01 levels of significance per experiment (29). All group values are given as means \pm SE. The SEs plotted in the figures are calculated separately within each subgroup. Two-tailed *P* values are presented. The Mann-Whitney U-test was used to test between two independent groups. Wilcoxon's matched-pair signed-rank test was used to test between paired incubations with and without addition of progesterone or testosterone (30).

RESULTS

Release of Progesterone. Malignant tumors released significantly more progesterone into the incubation medium than benign tumors or control ovaries ($P < 0.05$ for both, LSDT). Benign tumors did not release more progesterone than did control ovaries. Hormone concentrations in the medium of control ovaries, malignant and benign tumors (both "tumor" and "tumor base" stations), are shown in Fig. 1 and Table 1. In the cancer cases more progesterone was released from the "tumor" station than from the "tumor base" ($P < 0.03$, LSDT). In benign tumors no significant difference in concentration was noted among the three stations. Progesterone release from the contralateral ovary in tumor patients did not differ from that found in controls.

Release of Androstenedione. Significantly more androstenedione was released from malignant tumors than from benign tumors or control ovaries (Fig. 2) ($P < 0.05$ for both, LSDT). Hormone concentrations for all three stations are shown in Table 1. There was no difference in androstenedione concentration between the two stations "tumor" and "tumor base." Due to a low number of observations of contralateral ovaries no statistical comparison was made between contralateral ovaries and the other tissue stations (Fig. 2).

³ The abbreviations used are: LSDT, least significant difference test; EGF, epidermal growth factor.

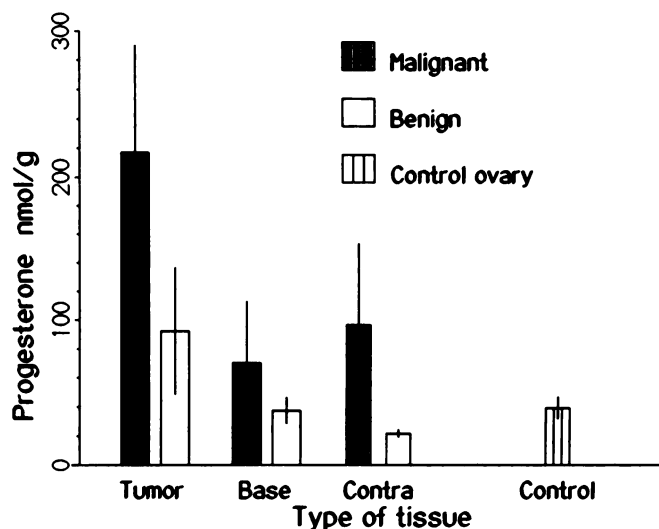


Fig. 1. Mean \pm SE (nmol/g wet weight) progesterone concentrations in media after 3-h incubations of malignant tumors ($n = 19$), benign tumors ($n = 14$), and tumor-free postmenopausal control ovaries ($n = 12$). Tissue of typical tumorous appearance (*Tumor*), tissue from just beneath the oviduct (*Base*), and tissue from the contralateral ovary (*Contra*) are shown separately.

Table 1 Mean \pm SE of steroid hormone concentrations (nmol/g tissue) in incubation media after 3-h incubations of tissue samples from vital tumor and control ovaries

	n	Malignant tumor	n	Benign tumor	n	Control ovaries
P ^a	19	220 \pm 73	14	92 \pm 44	12	39 \pm 7.5
A	10	270 \pm 140	7	46 \pm 15	12	18 \pm 4.1
T	9	11 \pm 0.90	7	27 \pm 6.9	6	6.3 \pm 2.2
E	19	16 \pm 9.6	14	8.4 \pm 4.9	12	3.4 \pm 0.80

^a P, progesterone; A, androstenedione; T, testosterone; E, estradiol.

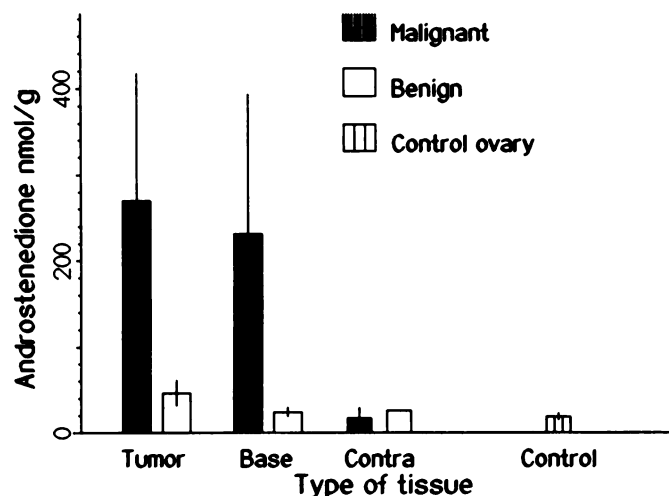


Fig. 2. Mean \pm SE (nmol/g wet weight) androstenedione concentrations in media after 3-h incubations of malignant tumors ($n = 10$), benign tumors ($n = 7$), and tumor-free postmenopausal control ovaries ($n = 12$). Tissue of typical tumorous appearance (*Tumor*), tissue from just beneath the oviduct (*Base*), and tissue from the contralateral ovary (*Contra*) are shown separately.

Release of Testosterone. Benign tumors released more testosterone than did malignant tumors or control ovaries (Table 1; $P < 0.05$ for both, LSDT). There was no difference in testosterone concentration in medium from the three stations "tumor," "tumor base," and contralateral ovary in benign or malignant cases (Fig. 3). The contralateral ovary, both malignant and benign, released more testosterone than did controls ($P < 0.05$ and 0.03, respectively, LSDT) (Fig. 3).

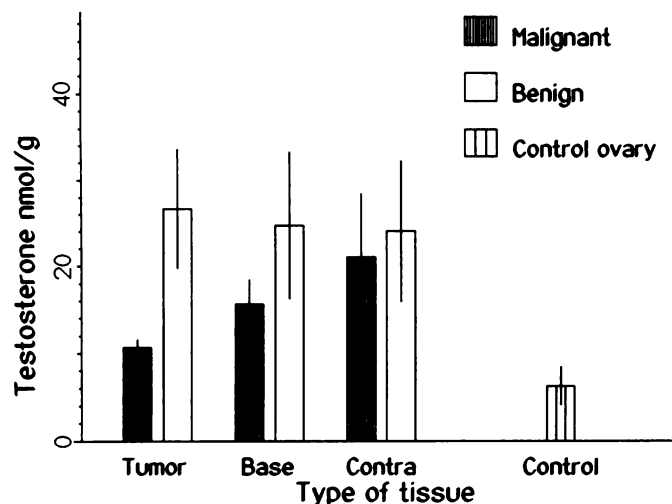


Fig. 3. Mean \pm SE (nmol/g wet weight) testosterone concentrations in media after 3-h incubations of malignant tumors ($n = 9$), benign tumors ($n = 7$), and tumor-free postmenopausal control ovaries ($n = 6$). Tissue of typical tumorous appearance (*Tumor*), tissue from just beneath the oviduct (*Base*), and tissue from the contralateral ovary (*Contra*) are shown separately.

Release of Estradiol. There was no significant difference in estradiol release between control ovaries, with malignant and benign tumors (Table 1). There was no difference between tissue from the different parts in each tumor, or between contralateral ovaries in tumor cases and controls. However, in some individual incubations the release of estradiol was substantial, while in others very little release occurred.

Postmenopausal-Premenopausal. Steroid hormone release from all different tumor stations, malignant and benign, was compared between premenopausal and postmenopausal women. No differences were found. The results from the postmenopausal group were in accordance with the results for the total material, *i.e.*, a tendency (but not significant) toward higher progesterone release from malignant tumors and significantly higher testosterone release from benign tumor tissue as compared with control ovaries: $27 (\pm 8.2)$ nmol/g ($n = 6$) versus $6.3 (\pm 2.2)$ nmol/g ($n = 6$) ($P < 0.02$, Mann-Whitney U-test). In the premenopausal cases, a tendency toward higher concentrations of progesterone from malignant tumors was also noted.

Stage and Grade. Moderately differentiated malignant tumors released more progesterone than did poorly differentiated malignant tumors: $250 (\pm 110)$ nmol/g ($n = 8$) versus $12 (\pm 2.2)$ nmol/g ($n = 6$) ($P < 0.002$, Mann-Whitney U-test). No differences in concentrations of androstenedione, testosterone, or estradiol were noted between tumors of different grades. Tumors of stages I and II released significantly more progesterone, androstenedione, and testosterone than tumors of stages III and IV: for progesterone, 240 ± 140 nmol/g ($n = 7$) versus 74 ± 61 nmol/g ($n = 7$) ($P < 0.05$); for testosterone, 12 ± 0.7 nmol/g ($n = 4$) versus 7.6 ± 1.1 nmol/g ($n = 3$) ($P < 0.05$); for androstenedione, 800 ± 570 nmol/g ($n = 2$) versus 18 ± 11 nmol/g ($n = 5$) ($P < 0.05$) (mean \pm SE; Mann-Whitney U-test).

Borderline Tumors. In the statistical analyses when borderline tumors were excluded from the malignant tumor group, the results were in accordance with the results obtained when borderline tumors and invasive carcinomas were analyzed together.

Incubation with Added Progesterone or Testosterone. In five control cases incubations were performed with and without the addition of testosterone or progesterone. The addition of progesterone doubled the release of androstenedione and increased the estradiol concentrations 20-fold but did not affect the release of testosterone. The addition of testosterone to incubation medium led to a 6-fold

increase in androstenedione concentration but did not affect the levels of estradiol (Table 2).

DISCUSSION

More progesterone and androstenedione were released from the tumorous ovary than from normal control ovaries. No differences in estradiol release were noted between tumorous and control ovaries. Malignant tumors released more progesterone and androstenedione than did benign tumors. On the contrary, testosterone concentrations were higher in medium from benign than from malignant tumors. Different parts of the tumor differed in hormone release: tumor tissue from malignant tumors released significantly more progesterone than did tumor base tissue (which contained more stromal cells).

Previous studies have shown that a high plasma concentration of progesterone parallels tumor volume (1). This investigation demonstrated a high release of progesterone, particularly from malignant tumors. Malignant tumor tissue released more steroids per mg tissue than benign tumor tissue or postmenopausal ovaries. This indicates that steroid release was not only related to tumor volume but also that malignant tissue was more active in releasing steroids. The difference between malignant and benign tissue was pronounced for progesterone release, an interesting finding since previous reports reveal prognostic information from plasma concentrations of progesterone (9, 10). The present results support the hypothesis that the elevated plasma levels of steroid hormones originate from the tumorous ovary and are not due to increased production by the adrenals. This is further supported by low serum concentrations of steroids in women with malignant disease of nonovarian origin (9).

In this study tissue samples consisted of a mixture of stromal and tumor cells. Tissue from areas of "typical tumorous appearance" contained more tumor cells than stromal cells, and "tumor base" tissue contained more stromal than tumor cells. This investigation could not determine the site (cell type) of hormone production. The results showed a higher release of progesterone from biopsies with more tumor cells than stromal cells. However, the biopsies from tumors of low grade released less than tumors of high grade. One explanation could be that a tumor of low grade contains fewer stromal cells than tumors of higher grade. The tumor cells might produce substances that exert a paracrine stimulation of progesterone production from the stromal cells. Since biopsies from the tumor base contained fewer tumor cells, fewer stromal cells might have been activated, resulting in a low release of progesterone. In low-grade tumors the absolute number of stromal cells is low, and that could be the reason for a lower progesterone release.

If the high steroid production in tumors is not inherent, then what stimulates the steroid production of ovarian tumors? In earlier studies of ovarian carcinoma, plasma concentrations of follicle-stimulating and luteinizing hormone have been shown to be decreased in women with large tumor volume and increased steroid production, indicating a negative feedback (31). Simultaneously, however, elevated levels of

Table 2. Mean \pm SE of steroid hormone concentrations (nmol/g tissue) in incubation media of 5 postmenopausal control ovaries without (no addition) and with addition of progesterone (100 ng/incubation) or testosterone (100 ng/incubation)

	Androstenedione (nmol/g)	Testosterone (nmol/g)	Estradiol (nmol/g)
No addition	11 ± 2.3	3.6 ± 1.1	7.4 ± 1.4
Progesterone addition	21 ± 2.3^a	4.0 ± 0.7	128 ± 62^a
Testosterone addition	66 ± 13.5^a		12 ± 2.2

^a $P < 0.001$.

human chorionic gonadotropin are noted, and human chorionic gonadotropin could be a stimulating factor of steroid production (29). Mac Donald *et al.* (20) suggest that ovarian tumors produce a growth-promoting factor that induces hyperplasia of the stroma, while the increased luteinizing hormone levels normally present in postmenopausal women stimulate steroidogenesis. Possible stimulators are EGF or transforming growth factor α . EGF plays a role in regulating ovarian cancer cell growth (32), and patients with EGF receptor-positive ovarian carcinomas demonstrate a better clinical follow-up (33). Interaction between EGF and steroid hormone production in rat granulosa cells has been demonstrated in several studies (34, 35). In breast carcinomas an inverse correlation exists between EGF receptor and steroid hormone status (36). Studies are in progress that are investigating the possibly stimulatory role of EGF/transforming growth factor α in steroid production of ovarian carcinoma and its relationship to ploidy level of the tumor.

From our results it is obvious that ovarian tumor tissue has the ability to produce steroids *in vitro* without the addition of precursors. Following the addition of progesterone an increase in estradiol release in particular was noted, indicating a further metabolism of progesterone.

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