Gonadotropins and female sex steroid hormones in cyst fluid and serum from patients with ovarian tumors

C.M.G. Thomas[1, 2, 4], E.A. Boss[2, 3], H. Boonstra[2], D. van Tienoven[1], C.G.J. Sweep[1], L.F.A.G. Massuger[2]

[1]Departments of [1]Chemical Endocrinology and [2]Obstetrics and Gynecology, Radboud University Nijmegen Medical Centre, Nijmegen, [3]Current address: Department of Obstetrics and Gynecology, Máxima Medical Centre, Veldhoven (The Netherlands)

Summary

The objective of the present study was to determine the concentrations of LH, FSH, 17ß-estradiol and progesterone in ovarian cyst fluid and serum from patients with benign and malignant ovarian tumors and to assess the correlation of the gonadotropin and female sex steroid hormone concentrations with menopausal and tumor status. Ovarian cyst fluid and blood samples were prospectively collected from 103 patients with ovarian tumors. Seventy-four of the patients had benign ovarian tumors while 29 patients had malignant ovarian tumors. Malignant ovarian tumors showed significantly higher LH and FSH cyst fluid concentrations compared to concentrations from patients with benign tumors. Within the malignant subset, LH and FSH concentrations correlated with increasing FIGO stage and grade. Furthermore, LH and FSH cyst fluid concentrations showed strong correlations ($r > 0.62$) with serum concentrations in case of malignant tumors, especially in postmenopausal women, but not in case of benign tumors. The highest gonadotropin concentrations were observed in cyst fluid from malignant ovarian tumors. The most probable explanation for this is an increased vascular permeability within the cysts. Supportive evidence for such an increased vascular permeability is our previous finding of significantly higher VEGF concentrations in cyst fluid from malignant ovarian tumors. The possibility of ectopic production of LH and FSH by malignant ovarian tissue cannot completely be ruled out..

Key words: Gonadotropins; Female sex steroids; Ovarian tumor; Cyst fluid; Menopausal status.

Introduction

The majority of epithelial ovarian tumors, benign as well as malignant, present as cystic structures, with or without solid components [1]. Ovarian cysts contain variable amounts of fluid, most probably secreted by the ovarian surface epithelium (OSE) cells [2]. The macroscopic characteristics of cyst fluid reflect the differentiation of the OSE into one of the coelomic epithelium derivatives (serous, mucinous, endometrioid) [2-4]. There are only few studies on the determination of various analytes present in ovarian cyst fluid. A number of these studies involve measurement of gonadotropins or female sex steroid hormones [5-11]. Most of these studies focused on the differentiation between functional and neoplastic cysts. The studies that aimed to discriminate between benign and malignant tumors report conflicting results [7, 9-11].

The present study was designed to determine gonadotropin (LH, FSH) and female sex steroid hormone (17β-estradiol, progesterone) concentrations in cyst fluid and serum from patients with benign or malignant ovarian tumors, and to assess the correlation of these hormone levels with tumor and menopausal status and their relationship with known prognostic factors.

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Materials and Methods

Cyst fluid collection

One hundred and three patients with an ovarian tumor planned for surgical removal were informed about the background and objectives of the present study. After having given their informed consent these patients were included in the cyst fluid collection procedure in accordance with the guidelines of the ethical and institutional board of the Radboud University Nijmegen Medical Centre. Immediately after surgical removal, the tumor was transported to the pathology laboratory where aseptic fine needle aspiration was performed to collect cyst fluid. Next, the cooled fluid sample was transported to the Department of Chemical Endocrinology, and after centrifugation at 3000 x g for 10 min, the supernatant was collected and immediately stored at –35°C. From 61 of these patients blood was collected up to four weeks before surgery, centrifuged at 2000 x g for 15 min and the serum was stored at –35°C until assayed. A gynecologic pathologist performed the histopathologic diagnoses according to WHO criteria.

Immunoassays

Determination of LH and FSH concentrations in cyst fluid and serum was performed with the random access analyzer AxSym (Abbott Laboratories, Chicago, IL, USA), a fully automated analyzer system based on microparticle enzyme immunoassay (MEIA) technology. Cyst fluid and serum 17β-estradiol and progesterone concentrations were measured with in-house radioimmunoassay (RIA) procedures [12]. Prior to RIA, the samples were extracted twice with diethylether and the dried extracts subjected to Sephadex LH-20 chromatography for isolation of the 17β-estradiol and progesterone containing fractions. The minimum detectable concentrations of 17β-estradiol and progesterone were 75 pmol/l and 1.3 nmol/l, respectively. The precision in terms of within- and between-assay

coefficients of variation for means of duplicate determinations for 17β-estradiol was 4.3% and 7.9%, and 4.1% and 9.1% for progesterone. Reference values for gonadotropins and sex steroids in blood in our laboratory were for postmenopausal patients: 12-75 IU/l for LH; 37-140 IU/l for FSH; < 200 pmol/l for 17β-estradiol, and < 1.3 nmol/l for progesterone.

Statistics

Results are given individually as concentration and as median concentration and range (minimum-maximum). Assay results below the detection limits of the immunoassays for the different components in the clinical specimens were considered half of the detection limit of the assay. Significance of differences in concentrations of the various analytes between the histopathologic subgroups was tested using the Mann-Whitney-U test for independent samples (level of significance $p < 0.05$). Correlations between cyst fluid and serum concentrations were calculated using the Spearman rank correlation coefficient. The receiver operating characteristic (ROC) curve analysis was performed on LH and FSH cyst fluid concentrations determined in cyst fluid from benign and malignant ovarian tumors for calculating areas under the curve (AUC) and statistical significance for comparisons of various subsets according to histopathology or menopausal status.

Results

Table 1 presents the histopathologic results of 103 ovarian tumors from which 29 malignant and 74 benign ovarian cysts were aspirated for the collection of fluid. Three malignant cysts with metastatic localization in the ovary of a primary tumor elsewhere (two breast, one colon) were excluded from cyst fluid analysis. In up to 30 cases only a subset of the hormonal parameters could be analyzed due to the viscosity of the cyst fluids or too small sample volumes.

Table 1. — *Histopathological diagnosis of ovarian cysts and aspirated cyst fluids (n = 103).*

	N
Malignant	
serous cystadenocarcinoma	10
mucinous cystadenocarcinoma	8
endometrioid carcinoma	6
undifferentiated cystadenocarcinoma	2
metastatic*	3
Total malignant	29
Benign	
serous cystadenoma	18
mucinous cystadenoma	21
dermoid cyst	11
functional cyst	11
cystic endometriosis	13
Total benign	74

N = number of tumors in each group; * = excluded from cyst fluid analysis.

Cyst fluid gonadotropin and steroid concentrations according to menopausal status and histopathologic subtype

Figures 1A-D show the LH, FSH, 17β-estradiol, and progesterone concentrations as measured in cyst fluids from 45 premenopausal and 25 postmenopausal women. The 45 premenopausal women could further be subdivided into 37 women with benign tumors (4 histopathology subtypes) and eight women with malignant tumors (3 histopathology subtypes). The 25 postmenopausal women had benign tumors in 13 cases (2 histopathology subtypes) while the 12 other women had malignant ovarian cysts from four different histopathology subtypes.

The LH cyst fluid concentrations (Figure 1A) from all the benign tumors were invariably lower than 2.6 IU/l (except 3 cases) whereas more than half the number of all malignant cysts (13 out of 20) were higher than that concentration. After excluding the invariably low malignant mucinous cyst LH results (n = 6, significantly different from the other pre- and postmenopausal serous cyst LH and FSH results, $p < 0.05$), 13 out of the 14 malignant cysts were higher than 2.6 IU of LH per liter. Comparable results were found for FSH, with only three out of the 50 benign ovarian cyst fluids and half the number of all the malignant cysts higher than 16 IU/l (Figure 1B). Figures 1C and 1D show that such differences were not found in 17β-estradiol or progesterone cyst fluid concentrations between the benign and malignant subgroups.

Table 2 gives the results of ROC curve analysis for LH and FSH cyst fluid concentrations determined in cyst fluid from benign and malignant ovarian tumors. The AUCs for comparisons of all grouped subsets were equal or higher than 0.85 for LH and FSH (both $p < 0.0001$). After subdividing this data according to menopausal status, the AUCs for LH and FSH were respectively 0.75 and 0.88 for premenopausal women ($p < 0.05$), and 0.89 and 0.87 for postmenopausal women ($p < 0.002$). After excluding the malignant mucinous cyst results, the AUCs of the remaining dataset for LH and FSH increased to 0.99 and 0.95 (both $p < 0.0001$), whereas in the same order, the comparison of all the benign data with only

Table 2. — *ROC curve analysis for LH and FSH cyst fluid concentrations determined in cyst fluid from benign and malignant ovarian tumors; Areas under the curve (AUC) and statistical significance for comparisons of various subsets according to histopathology or menopausal status.*

Parameter	Benign No.	Malignant No.	AUC	95% CI	Significance p
All data					
LH	50	20	0.849	0.733-0.964	0.0001
FSH	50	20	0.884	0.805-0.963	0.0001
Premenopausal women					
LH	37	8	0.747	0.527-0.967	0.030
FSH	37	8	0.883	0.768-.998	0.001
Postmenopausal women					
LH	13	12	0.891	0.750-1.032	0.001
FSH	13	12	0.865	0.724-1.007	0.002
All benign versus malignant serous and others (mucinous excluded) *					
LH	50	14	0.994	0.982-1.006	0.0001
FSH	50	14	0.951	0.902-0.999	0.0001
All benign versus malignant mucinous-only *					
LH	50	6	0.508	0.309-0.708	0.947
FSH	50	6	0.728	0.580-0.877	0.070

* Because of the observed significant differences in cyst fluid gonadotropin concentrations of mucinous and the other histopathology types in malignant ovarian tumors, gonadotropin results were separately analyzed for subsets 'mucinous excluded' and 'mucinous-only'. No such concentration differences are present between histopathologic subsets of benign tumors

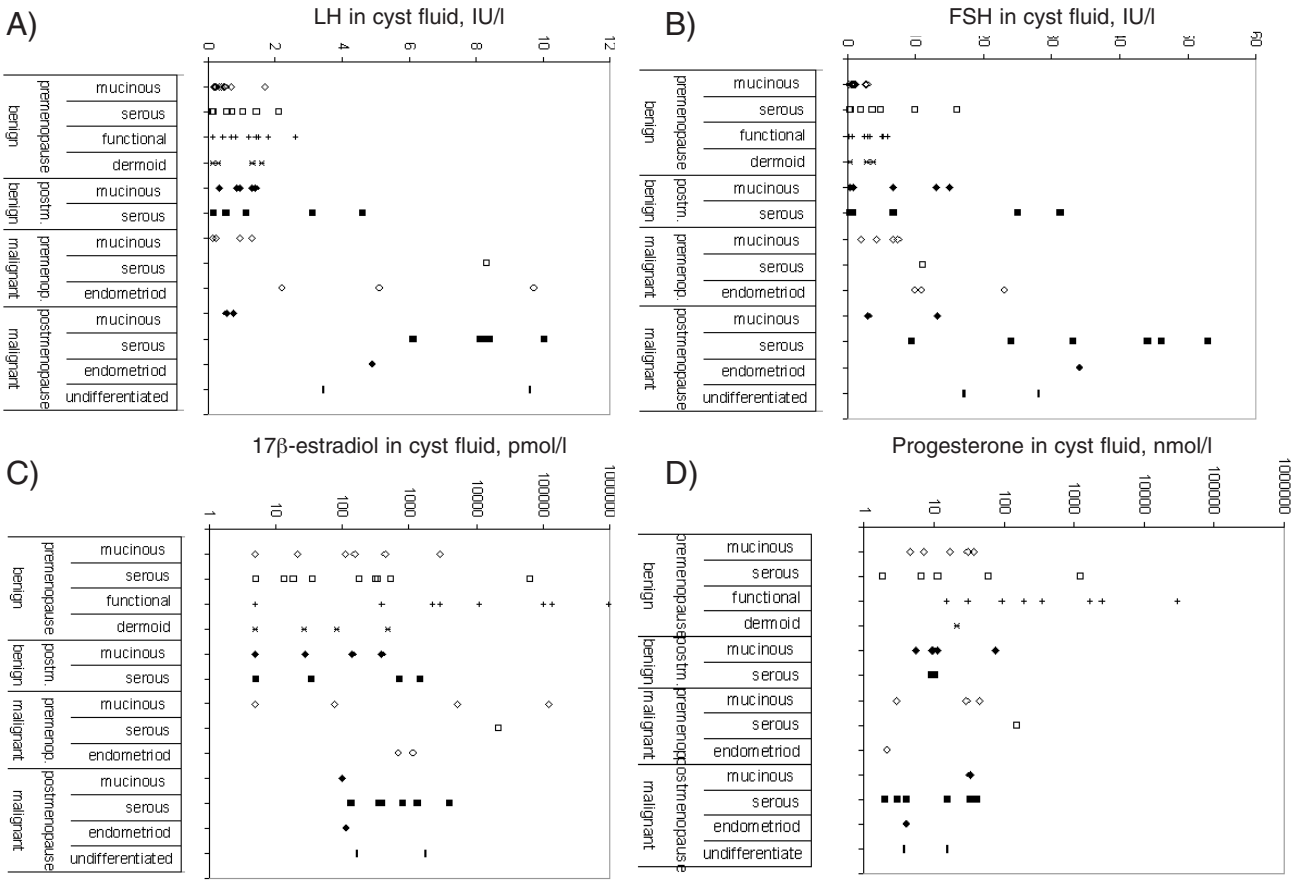

Figure 1. — Individual LH (A), FSH (B), 17ß-estradiol (C), and progesterone (D) concentrations in cyst fluid from ovarian tumors of different histopathologic origin.

those of the malignant mucinous cyst results gave non-significant AUCs of 0.51 and 0.73.

Correlation between cyst fluid and serum gonadotropin concentrations

Separately for both premenopausal and postmenopausal women the LH and FSH cyst fluid concentrations of the benign and malignant histopathologic subgroups were plotted against the corresponding LH and FSH serum concentrations (Figure 2). In both pre- and postmenopausal women with benign ovarian tumors, weak positive and negative correlations ranging between –0.128 and 0.284 were found for cyst fluid LH and FSH and their respective serum concentrations. With exception of the weak negative correlation between FSH in cyst fluid and serum from premenopausal women (r = -0.014), rather strong positive correlations (range 0.629-0.710) were found for cyst fluid LH and FSH and their respective serum concentrations in pre- and postmenopausal patients with malignant ovarian tumors. No such correlations could be observed in the case of 17β-estradiol or progesterone (data not shown).

Clinicopathologic characteristics

Table 3 presents gonadotropin and female sex steroid hormone cyst fluid concentrations related to tumor FIGO stage, histopathologic subgroup, and tumor differentiation grade. The median LH and FSH cyst fluid concentrations were significantly higher in FIGO Stages II, III, IV, malignant serous and histologic grade 2,3 as compared to Stage 1, malignant mucinous and grade 1, respectively. Although the median 17β–estradiol concentration was higher, and that of progesterone lower in Stage II, III, IV, malignant serous and grade 2, 3 as compared to those of stage 1, malignant mucinous and grade 1, respectively, these differences were not statistically significant.

Discussion

The present study intended to determine gonadotropins (LH, FSH) and female sex steroid hormones (17β-estradiol, progesterone) in cyst fluid from the different histopathology subgroups of ovarian tumors and to correlate these results with the clinical value. We observed significantly higher median LH and FSH cyst fluid concentrations in malignant compared to benign tumors. Reimer *et al.* also found higher FSH levels in malignant cysts [10]. However, in their study LH levels were found to be equal for benign and malignant cases. The inclusion in their study of four borderline tumors in the malignant group (n = 7) is a possible explanation for this difference.

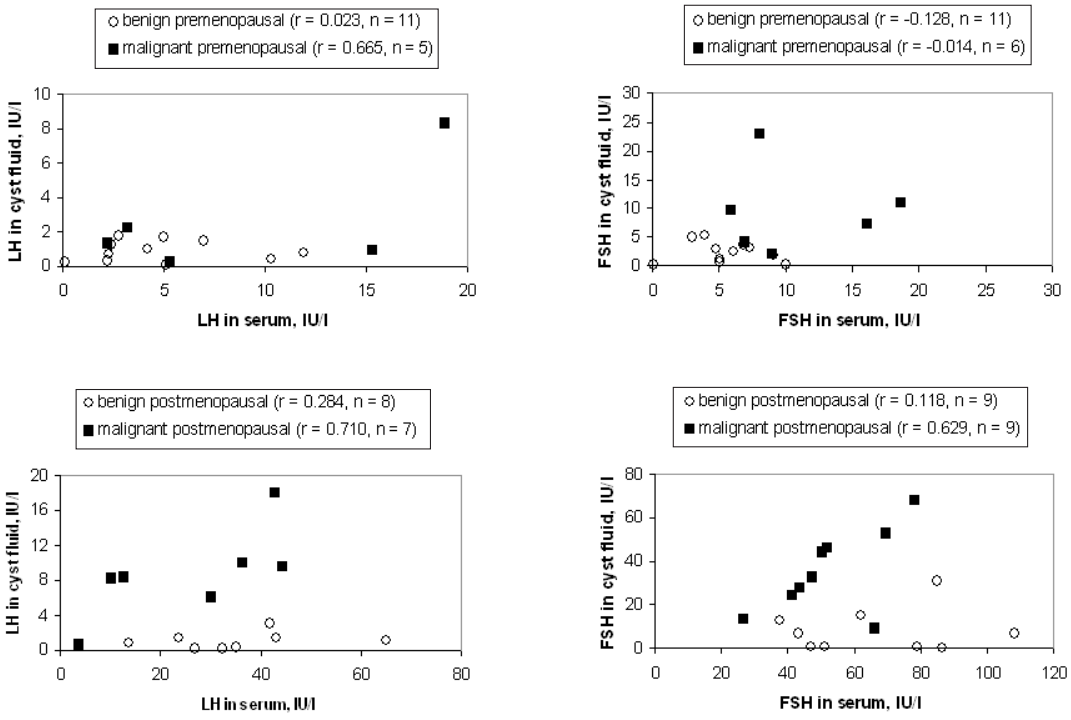

Figure 2. — LH and FSH cyst fluid concentrations in benign and malignant histopathologic subgroups of premenopausal and postmenopausal women as a function of the corresponding serum concentrations.

Table 3. — *Gonadotropin and steroid concentrations in cyst fluid related to histopathologic characteristics in malignant ovarian tumors.*

FIGO	LH	FSH	17β-estradiol	progesterone
Stage I	*IU/l*	*IU/l*	*pmol/l*	*nmol/l*
median	2.2	9.7	315	29
range	0.2-10	2.1-34	50-120000	0.6-84
N	11	11	14	13
Stage II-III-IV				
median	8.3[A]	28[B]	560	3.4
range	0.1-18	6.7-68	130-21000	1.9-150
N	9	9	9	9
Histology serous				
median	8.3[C]	39[D]	430	6.1
range	6.1-18	9.2-68	130-21000	1.9-150
N	8	8	9	9
Mucinous				
median	0.64	5.45	150	31
range	0.15-1.3	2.1-13.2	50-120000	3-44
N	6	6	7	6
Grade 1				
median	0.85	3.6	205	29
range	0.2-1.3	2.1-7.4	50-120000	4.2-84
N	4	4	6	5
Grade 2-3				
median	8.1[E]	23.5[F]	420	3.9
range	0.1-18	6.7-68	100-21000	0.6-150
N	16	16	17	17

[A, B] = Significantly higher ($p < 0.05$) cyst fluid concentrations were found in FIGO Stage II, III, IV compared to FIGO Stage I for LH and FSH; [C, D] = significantly higher ($p < 0.05$) cyst fluid concentrations were found in malignant serous compared to malignant mucinous tumors for LH and FSH; [E, F] = Significantly higher ($p < 0.05$) cyst fluid concentrations were found in histologic grade 2-3 compared to grade 1 tumors for LH and FSH.

Krämer *et al.* describe higher cyst fluid LH and FSH concentrations in the serous malignant than in the serous benign subtype [9]. In that study mucinous cyst fluids were excluded due to viscosity of the samples. Although we experienced similar problems with mucinous fluids, we were able to determine gonadotropin and steroid levels in both benign as well as malignant mucinous tumors. In benign tumors no differences were found for gonadotropin levels between both subtypes. However, significantly higher LH and FSH concentrations were found in cyst fluids from malignant serous compared to malignant mucinous tumors. To our opinion it seems unlikely that assay problems form the basis of this difference, as the difference was not noticed between the benign mucinous and serous cysts. The over-representation of higher tumor stages (in which higher gonadotropin concentrations were found) in the serous subgroup (2 FIGO Stage I and 8 FIGO Stage II, III, IV serous, compared to 7 FIGO Stage I and 1 FIGO Stage II, III, IV mucinous) probably is a better explanation for this finding. Higher LH and FSH cyst fluid concentrations in malignant tumors might result from local production of LH and FSH by the tumor tissue, although there are no studies describing LH or FSH production by tumor tissue epithelium. Differences in gonadotropin cyst fluid concentrations between malignant and benign tumors may also be the result of differences in vascular permeability. Due to increased angiogenesis with more permeable vessels in case of malignancy, higher serum LH and FSH concentrations (especially in postmenopausal women)

may passively diffuse from the serum into the cyst fluid compartment. This hypothesis is in line with the strong positive correlation in malignant tumors for LH and FSH cyst fluid concentrations and their respective serum concentrations. The indication of differences in vascular permeability between malignant and benign ovarian cysts is supported by significantly higher Vascular Endothelial Growth Factor (VEGF) levels we earlier found in cyst fluid from malignant tumors compared with benign tumors [13]. VEGF acts not only as an activator of angiogenesis, but also leads to increased vascular permeability.

Our study did not observe significant differences in 17β-estradiol and progesterone cyst fluid concentrations between the histopathologic subgroups. These results are in line with those from Gaetje and Popp who determined 17β-estradiol and progesterone in cyst fluid from ovarian tumors [7]. However, it contradicts the results of the study by Reimer et al. who reported significantly lower 17β-estradiol levels in cyst fluid from malignant tumors compared to those found in non-malignant cysts [10]. Ivarsson et al. describe production of 17β-estradiol and progesterone by normal ovarian surface epithelium cells in vitro from pre- as well as postmenopausal patients [14]. It is reasonable to assume that the finding of endogenous production of steroids by ovarian surface epithelium is a normal feature of epithelial cells that is still present during malignant transformation, rather than a tumor specific property [14]. Results from the present study indicate only limited potential prognostic value (in terms of correlation with FIGO stage or histologic grade) for cyst fluid gonadotropins or steroids in malignant tumors. Although significantly higher median cyst fluid LH and FSH concentrations were found in higher tumor stages and high-grade tumors, the results were too scattered to draw conclusions for individual patients.

Progesterone levels tended to be higher in fluid from prognostically more favorable tumors (lower stage and better histologic grade). Other studies describe a more favorable prognosis for tumors positive for the progesterone receptor compared to those that did not express this receptor [15, 16]. However, the relationship between tumors that are positive for the progesterone receptor and their cyst fluid progesterone levels remains unclear as well as their impact on prognosis, and might be the subject of future studies. In conclusion, higher gonadotropin concentrations were found in cyst fluid from malignant tumors compared to those from benign tumors. The correlation of cyst fluid and serum gonadotropins in malignant tumors might be explained by increased vascular permeability. Future studies have to determine whether the differences in ovarian cyst fluid gonadotropin concentrations might be of clinical interest. The therapeutic use of LHRH agonists in the treatment of ovarian cancer has been suggested [17, 18]. Further exploration of the efficacy of such a treatment has to be performed to find out whether cyst fluid gonadotropin levels might be of additional value in determining which proportion of the patients may benefit from such a treatment.

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Address reprint requests to:
C.M.G. THOMAS, Ph.D.
Department of Chemical Endocrinology,
479 ACE, Radboud University Nijmegen
Medical Centre, P.O. Box 9101,
6500 HB Nijmegen (The Netherlands)
e-mail: c.thomas@ace.umcn.nl