CLINICAL STUDY

Inhibin A, B and pro- α C in serum and peritoneal fluid in postmenopausal patients with ovarian tumors

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Abstract

Objective: To compare serum and peritoneal fluid concentrations of inhibin A, B, and pro- α C in women with ovarian tumors.

Methods: Serum and peritoneal fluid samples were taken from 41 postmenopausal women operated on for an ovarian tumor. Twenty-one patients with endometrial cancer formed a control group. Serum and peritoneal fluid inhibin A, B, and pro- α C concentrations, and serum FSH and tumor marker CA 125 (study group only) concentrations were analyzed.

Results: Inhibin A was found in low concentrations (median 4.1 pg/ml, range < 2–29 pg/ml) in serum in most postmenopausal patients with epithelial ovarian carcinoma, whereas inhibin B was not measurable. Inhibin pro- α C circulated in high concentrations (median 125 pg/ml, range 37–>1000 pg/ml). All inhibins were found in clearly greater concentrations in the peritoneal fluid than in serum. International Federation of Gynecology and Obstetrics (FIGO) stage III–IV and poor differentiation grade were associated with significantly lower concentrations of inhibin A and pro- α C in the peritoneal fluid compared with stages I–II or low grade. This correlation was not found in the serum concentrations of inhibin A or pro- α C. In the control group, no dimeric inhibins were found in serum, and pro- α C circulated in median concentrations of 47 pg/ml (range 12–174 pg/ml).

Conclusions: Postmenopausal patients with epithelial ovarian tumors had low concentrations of inhibin A and relatively high concentrations of inhibin pro- α C in serum. The peritoneal fluid concentrations of all inhibins far exceeded those in the serum. Relatively low concentrations of inhibin A and pro- α C in the peritoneal fluid of patients with ovarian cancer seem to be associated with high stage and grade and, to a lesser degree, with positive peritoneal cytology.

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Introduction

Endogenous hormones, in particular pituitary gonadotropins, are believed to play a part in the pathogenesis of ovarian cancer (1). Inhibin, a glycoprotein hormone produced by the ovary and testis, was originally discovered on the basis of its ability to suppress pituitary secretion of follicle stimulating hormone (FSH) (2). Inhibins and activins belong to the transforming growth factor- β family of growth factors, which also have been implicated in ovarian tumor pathogenesis (3–6) by affecting the growth factor or the immune system (7). An imbalanced expression of inhibin and activin subunits has been purported to lead to uncontrolled epithelial proliferation (8, 9). Increased serum concentrations of total inhibin were first discovered in patients with ovarian granulosa cell tumors (10). High serum concentrations of total inhibin

were later measured also in patients with mucinous epithelial carcinomas of the ovary (11, 12), whereas relatively low concentrations have been detected in other types of epithelial ovarian carcinomas (11–14).

The newly developed enzyme-immunoassays have made it possible to measure specifically the serum concentrations of inhibin A, inhibin B, and the precursor of the α -chain, inhibin pro- α C. The characteristics of serum inhibin A and B during the normal menstrual cycle and after menopause have recently been investigated (15–17). The function of pro- α C has not yet been ascertained, but its serum concentrations are known greatly to exceed those of inhibins A and B (13, 18). Few data are available as to which molecular forms of inhibins are secreted into the circulation by different types of ovarian tumors (5, 11–14), and the role and function of inhibins in the peritoneal fluid have not yet been clarified (19). Recent studies suggest that

the α -subunit, rather than the dimeric forms, are increased in epithelial ovarian carcinomas, whereas ovarian granulosa cell tumors preferentially secrete dimeric inhibins (13, 14).

The aim of this study was to obtain information about concentrations of inhibin A, inhibin B, and inhibin pro- α C in serum and peritoneal fluid in postmenopausal women with ovarian tumors. In addition, serum concentrations of FSH and CA 125 were measured.

Patients and methods

Forty-one postmenopausal women operated on for an ovarian tumor at the Department of Obstetrics and Gynecology of Tampere University Hospital in 1995–1997 were included in this study. Thirty of them had a malignant and 11 had a benign tumor. A patient was classified as postmenopausal if she had been amenorrheic for at least 1 year. The median age was 67 years (range 50–85 years), and the mean interval since the last menstrual period was 16.4 years (range 3–31 years). The malignant tumors included 27 epithelial carcinomas (15 serous, three mucinous, four endometrioid, three clear cell and two undifferentiated), one granulosa cell tumor, and two metastatic carcinomas to the ovary (originally a mammary carcinoma and a Krukenberg tumor). Two of the malignant tumors were of low malignant potential ('borderline'). The benign tumors included five serous and three mucinous cystadenomas, two fibromas and one mature teratoma.

Twenty-one postmenopausal women operated on for endometrial carcinoma formed a control group. No patient had received preoperative radiation therapy. The carcinomas included 18 endometrial adenocarcinomas, one clear cell carcinoma and two mixed mesodermal tumors of the endometrium. Hormone replacement therapy had been used by three patients, but it had been discontinued for at least 3 weeks before the operation. A total of 12 patients had International Federation of Gynecology and Obstetrics (FIGO) stage I, six had stage II, two stage III and one patient had stage IV disease. No myometrial invasion was found in seven patients, invasion to less than half of the depth of the myometrium was present in nine patients, and five patients had a deep myometrial invasion. The carcinoma was well differentiated in seven patients, and 14 patients had a moderately or poorly differentiated carcinoma.

Fifteen milliliters of blood from a cubital vein were drawn 1–2 h before operation. Serum was separated and stored at -20°C until required for assay for FSH, CA 125 (for ovarian tumors only), inhibins A, B, and pro- α C. The patients were operated on under general halothane anesthesia. At laparotomy, immediately after the abdominal cavity was penetrated, peritoneal fluid was collected from the pouch of Douglas, and then centrifuged for 10 min at 1850 *g*. The supernatant was frozen at -20°C until required for assay for inhibins A, B and pro- α C. Another sample was taken for cytological

examination. The study procedure was approved by the Ethics Committee of Tampere University Hospital. Informed consent was obtained from the patients after the purpose and nature of the study had been fully explained to them.

Tissue samples and samples for peritoneal cytology obtained during surgery were fixed in formalin and ethanol, respectively, and processed according to standard histological and cytological methods.

Assays for inhibins A, B and pro- α C

Concentrations of inhibins A, B, and pro- α C were measured using commercially available ELISA kits (Serotec Ltd, Oxford, UK) according to the manufacturer's instructions. Intra- and interplate coefficients of variation, as given by the manufacturer, were less than 10% for inhibin A, and less than 7% for inhibin B; the manufacturer does not give coefficients of variation for pro- α C. The sensitivity of the assay was 2 pg/ml for inhibin A, 10 pg/ml for inhibin B (20) and 2 pg/ml for inhibin pro- α C. A dilution of 1:5 was used for inhibins A and B, and 1:15 for the pro- α C in serum and peritoneal fluid samples. The upper measurable limits were 1000 pg/ml for inhibins A, B, and pro- α C.

Assays for FSH and CA 125

FSH was determined by an AutoDELFIA immunoanalyzer using AutoDELFIA hFSH B107–101 (Wallac Ltd, Turku, Finland). As given by the manufacturer, the range of measurements possible was 0.2–256 U/l, and the intra- and interassay coefficients of variation at 34.1 and 31.0 U/l were 2.3 and 3.1%, respectively. FSH concentrations greater than 30 U/l were considered as postmenopausal. CA 125 analysis was performed by CobasCore CA 125 enzyme-immunoassay analysis kit (Roche, Basel, Switzerland). As given by the manufacturer, the sensitivity of the assay was <1 U/l, and the intra- and interassay variations were less than 5.3 and 7.5%, respectively. CA 125 concentrations exceeding 35 U/l were considered to be increased.

Statistics

Statistical analysis was performed using SPSS Statistical Software for Windows version 7.5. Non-parametric tests were used because of the non-Gaussian distribution of values. Differences in hormone concentrations between groups were tested with Kruskal–Wallis and Mann–Whitney *U*-tests. Spearman's correlation test was used as appropriate. Concentrations below the limits of detection were considered to be zero in the statistical analyses. Concentrations exceeding the upper limits or 1000 pg/ml of the inhibin assays were approximated as values of 1001 pg/ml in order to enable the statistics to run with continuing parameters. *P* values <0.05 were considered to be statistically significant.

Table 1 Serum and peritoneal fluid concentrations of the inhibins (pg/ml) in 26 postmenopausal women with serous or mucinous epithelial ovarian tumors. Data are given as median with ranges.

	Inhibin A	Inhibin B	Inhibin pro- α C
Serous tumors ($n = 20$)			
Serum	4.1 (<2–23)	<10 (<10–100)	113 (43–290)
Peritoneal fluid	24 (4–88)	48 (<10–>1000)	377 (68–>1000)
$P^\dagger$	0.000	0.047	0.001
Mucinous tumors ($n = 6$)			
Serum	<2 (<2–29)	<10 (<10–21)	305 (64–732)
Peritoneal fluid	156 (81–685)	105 (41–384)	>1000 (23–>1000)
$P^\dagger$	0.018	0.068	0.046

† Serum compared with peritoneal fluid.

Results

The results regarding inhibins in serous and mucinous tumors are presented in Table 1. Both serous and mucinous tumors secreted low concentrations of inhibin A into the circulation, with no significant difference between them. Only two patients had measurable inhibin B in serum. One was a 75-year-old woman with stage IV serous adenocarcinoma with a serum concentration of inhibin B 100 pg/ml. The other (inhibin B 21 pg/ml) had a semimalignant mucinous cystadenoma. Serum pro- α C concentrations were high in both mucinous and serous ovarian tumors, with no significant difference between the groups. The serous tumors were associated with greater concentrations of CA 125 and FSH than the mucinous tumors (CA 125 median 786, range 33–22150 U/l and median 24, range 8–134 U/l, respectively; FSH median 39, range 2.6–90 U/l and median 10, range 3–40 U/l, respectively).

The concentrations of the inhibins associated with malignant or benign histology are presented in Table 2. Low serum concentrations of inhibin A were found in patients with malignant ovarian tumors, but no measurable concentrations were found in the case of benign tumors. Inhibin B was not measurable, except for the two patients discussed above. Pro- α C concentrations were equally high in both groups. Malignant

tumors secreted significantly more CA 125 into the serum than did the benign ones (median 542, range 11–22150 U/l vs median 24, range 8–424 U/l, respectively). FSH concentrations were similar in both groups (median 35, range 2.6–90 U/l and median 31, range 5–40 U/l, respectively).

All inhibins studied were found in clearly greater concentrations in the peritoneal fluid than in serum (Tables 1, 2). Patients with mucinous tumors had significantly greater concentrations of inhibin A and inhibin pro- α C in the peritoneal fluid than did those with serous tumors ($P = 0.000$ for inhibin A, and $P = 0.047$ for inhibin pro- α C; Table 1). The concentration of inhibin A in the peritoneal fluid was lower in women with malignant tumors than in those with benign tumors ($P = 0.023$, Table 2).

The carcinoma patients with poor prognostic indicators or advanced stage, high grade, and positive peritoneal cytology had relatively low concentrations of inhibin A and pro- α C in their peritoneal fluid (Table 3). However, no correlation with prognostic factors was seen in the case of serum concentrations of inhibin A and pro- α C (data not shown).

Six postmenopausal women with non-epithelial or metastatic ovarian tumors were excluded from the statistical analysis. Three of them had stromal tumors (two fibromas, one granulosa cell tumor), one a benign

Table 2 Serum and peritoneal fluid concentrations of the inhibins (pg/ml) in 35 postmenopausal women with malignant or benign epithelial ovarian tumors. Data are given as median with ranges.

	Inhibin A	Inhibin B	Inhibin pro- α C
Malignant epithelial tumors ($n = 27$)			
Serum	4.1 (<2–29)	<10 (<10–>100)	125 (37–>1000)
Peritoneal fluid	29 (4.3–209)	56 (<10–>1000)	563 (68–>1000)
$P^\dagger$	0.001	0.002	0.001
Benign epithelial tumors ($n = 8$)			
Serum	n.d.	n.d.	145 (69–491)
Peritoneal fluid	85 (69–685)	90 (41–384)	>1000 (23–>1000)
$P^\dagger$	0.043	0.109	0.046

n.d., not detectable.

† Serum compared with peritoneal fluid.

Table 3 Association of peritoneal fluid inhibin A and pro- α C with peritoneal fluid cytology, FIGO stage, and tumor grade in patients with malignant ovarian epithelial tumors ($n = 27$). Data are given as median with ranges.

Prognostic indicator	Peritoneal fluid inhibin A		Peritoneal fluid inhibin pro- α C		P
Peritoneal cytology					
Negative ($n = 12$)	127	(6–209)	>1000	(68–>1000)	Inhibin A: 0.059
Positive ($n = 15$)	23	(4–87)	322	(74–>1000)	Inhibin pro- α C: 0.047
FIGO stage					
I–II ($n = 9$)	137	(117–209)	>1000	(68–1000)	Inhibin A: 0.000
III–IV ($n = 18$)	23	(4–87)	304	(68–>1000)	Inhibin pro- α C: 0.000
Grade					
1 ($n = 6$)	146	(117–209)	>1000		Inhibin A: 0.001
2–3 ($n = 21$)	24	(4–232)	349	(59–>1000)	Inhibin pro- α C: 0.002

teratoma and two had a metastatic carcinoma to the ovary. The patient with the granulosa cell tumor had exceptionally high inhibin B concentrations compared with all other patients. Her inhibin B concentrations were >1000 pg/ml in serum and >1000 pg/ml in the peritoneal fluid, whereas those of inhibin A and pro- α C were 71 pg/ml and 343 pg/ml in serum, and 414 pg/ml and >1000 pg/ml in the peritoneal fluid.

In the control group, no measurable inhibin A or inhibin B was found in serum, except in two patients. One patient with a very low serum concentration of inhibin A (5.8 pg/ml) was a 67-year-old woman with a moderately differentiated endometrial adenocarcinoma stage I A; the other patient with a serum inhibin B concentration of 99 pg/ml was a 55-year-old woman, also with a moderately differentiated stage I A endometrial adenocarcinoma. Inhibin pro- α C circulated in a concentration of 47 pg/ml (range 12–174 pg/ml). Although unmeasurable in serum, some inhibin A was found in the peritoneal fluid (median 15, range <2–89 pg/ml). Inhibin pro- α C was found in significantly greater concentrations in the peritoneal fluid than in serum (median 465, range 119–>1000 pg/ml in peritoneal fluid; $P = 0.043$). No significant differences were found when peritoneal fluid concentrations of inhibin A or pro- α C were correlated to prognostic factors or stage, grade, myometrial invasion, and peritoneal cytology.

Discussion

Inhibin A and B are produced by the ovary and secreted into the circulation in fluctuating concentrations throughout the menstrual cycle. Inhibin B appears to be unmeasurable after menopause, because of the cessation of ovarian function (15–17), whereas low levels of inhibin A secretion (in the range of 4–20 pg/ml) have been demonstrated even in healthy postmenopausal women (13). In addition, we have previously found that postmenopausal women with uterine leiomyomas or benign ovarian cysts have low (<2–13 pg/ml) serum

concentrations of inhibin A (17). In the present control group of endometrial carcinoma patients with healthy ovaries, one had 5.8 pg/ml of inhibin A in the serum. The greatest concentration of inhibin A encountered in the present study group was 29 pg/ml in a patient with a mucinous cystadenocarcinoma and, overall, only six of 27 patients (22%) with malignant ovarian tumors had inhibin A concentrations exceeding 13 pg/ml. We could not find any correlation between serum inhibin A concentrations and the extent of the disease; Lamber-Messerlian *et al.* (13) also found serum inhibin A concentrations to be similar in both postmenopausal ovarian cancer patients and healthy controls. In contrast, a recent study has suggested that an increased (>1.21 pg/ml) preoperative serum level of inhibin A is associated with poor prognosis (21). However, in all these studies, the concentrations of inhibin A have mostly been so close to the limit of detection or 2 pg/ml that the reliability of the results may have been affected. It can be concluded that inhibin A does not seem to be an important serum marker for epithelial ovarian cancer.

The present finding that inhibin B was detectable in the serum of only occasional postmenopausal women patients with epithelial ovarian tumors is in agreement with the findings of previous studies (13, 22). It may be that serum inhibin B reflects the presence of functioning granulosa cells, either normal (15–17) or transformed as a result of a pathologic process affecting the granulosa cells (24, 25), and therefore is not detectable in the serum of postmenopausal patients with epithelial ovarian carcinomas. Patients with ovarian granulosa cell tumors have been demonstrated to have exceptionally high concentrations of both inhibin B and inhibin A (25). Unfortunately, the present study included only one patient with a granulosa cell tumor, but she did have very high serum concentration of inhibin B (>1000 pg/ml), a moderately increased concentration of inhibin A (71 pg/ml), and a very low concentration of FSH (0.2 U/l).

The origin of inhibin pro- α C in women is believed to be the ovary (23), although the adrenal gland may

also be involved (22). In contrast to inhibins A and B, pro- α C is clearly detectable in serum in the range of 40–50 pg/ml even after menopause (13, 18). Our controls or postmenopausal patients with endometrial carcinoma had similar (median 47 pg/ml) pro- α C serum concentrations. Inhibin pro- α C has been suggested as a serum marker for epithelial ovarian carcinoma (13). In the present study group, serum inhibin pro- α C concentrations were found to be 2–10-fold greater than the concentrations reported in healthy postmenopausal women (13, 18) and in postmenopausal patients with endometrial cancer. Median pro- α C concentrations were 125 pg/ml (range 37–>1000 pg/ml) in patients with ovarian carcinoma, and 21 of 27 patients with ovarian carcinoma (78%) had serum pro- α C concentrations exceeding 47 pg/ml. Surprisingly, no difference in serum pro- α C concentrations was found when comparing benign and malignant tumors. Because of the small number of patients ($n=8$) with benign tumors, no definite conclusions can be drawn. However, a recent study suggested that a new α -inhibin-directed assay (α C IFMA), when combined with measurement of CA 125, would increase the rate of detection of ovarian cancer (26).

In the present study, the peritoneal fluid concentrations of all inhibins far exceeded the corresponding serum values. High peritoneal fluid concentrations of both total inhibin and dimeric inhibin have previously been measured in fertile-aged healthy women and in those with endometriosis (19, 27, 28). Little, if anything, is known about peritoneal fluid concentrations of inhibins in postmenopausal women, and no normal range has been determined previously. In our control group, no inhibin B and only little inhibin A was demonstrated; in contrast, the peritoneal fluid pro- α C concentrations almost equalled those measured in the study group, although the serum concentrations in the control group were not greater than reported previously (18). Unfortunately, postmenopausal healthy women do not normally have sufficient peritoneal fluid to be collected, so that it is very difficult to obtain information about peritoneal inhibin concentrations after menopause in general.

Interestingly, in the case of ovarian carcinomas, indicators of poor prognosis such as high stage or grade and positive peritoneal cytology were associated with relatively low peritoneal fluid inhibin A and pro- α C concentrations. The control group included only one patient with stage IV disease, but she did have a low peritoneal fluid concentration (120 pg/ml) of inhibin pro- α C. A previous study has suggested that the loss of α -inhibin expression in epithelial ovarian carcinoma may be a sign of an early mechanism leading to increased growth potential (8), and thus the inhibin α -subunit may have tumor-suppressing properties (29). The present findings of decreased inhibin pro- α C in the peritoneal fluid of patients with progressive ovarian carcinoma support this theory.

In conclusion, epithelial malignant ovarian tumors were associated with detectable, albeit low, serum concentrations of inhibin A in postmenopausal women, whereas inhibin B was undetectable. Inhibin pro- α C circulated in high concentrations in serum, with no clear association with any type of tumor, both in benign and in malignant ovarian tumors. There was a concentration gradient between peritoneal fluid and serum for all forms of inhibins studied. Moreover, it is possible that low concentrations of inhibin A and pro- α C in the peritoneal fluid reflect a poor prognosis for malignant epithelial ovarian tumors.

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References

- 1 Helzlsouer KJ, Alberg AJ, Gordon GB, Longcope C, Bush TL, Hoffman SC *et al.* Serum gonadotropins and steroid hormones and the development of ovarian cancer. *Journal of the American Medical Association* 1995 **274** 1926–1930.
- 2 Burger HG. Inhibin. *Reproductive Medicine Review* 1992 **1** 1–20.
- 3 Burger HG & Fuller PJ. The inhibin/activin family and ovarian cancer. *Trends in Endocrinology and Metabolism* 1996 **7** 197–202.
- 4 Wallace EM. Inhibins: new tumour markers in gynaecology. *Tumour Marker Update* 1996 **8** 105–110.
- 5 Burger HG, Robertson DM, Cahir N, Mamers P, Healy DL, Jobling T *et al.* Characterization of inhibin immunoreactivity in postmenopausal women with ovarian tumours. *Clinical Endocrinology* 1996 **44** 413–418.
- 6 Burger HG. Inhibin as a tumour marker. *Clinical Endocrinology* 1994 **41** 151–153.
- 7 Petraglia F. Inhibin, activin and follistatin in the human placenta – a new family of regulatory proteins. *Placenta* 1997 **18** 3–8.
- 8 Zheng W, Luo MP, Welt C, Lambert-Messerlian G, Sung JC, Zhang Z *et al.* Imbalanced expression of inhibin and activin subunits in primary epithelial ovarian cancer. *Gynecologic Oncology* 1998 **69** 23–31.
- 9 Petraglia F, Florio P, Luisi S, Gallo R, Gadducci, Vigano P *et al.* Expression and secretion of inhibin and activin in normal and neoplastic uterine tissues. High levels of serum activin A in women with endometrial and cervical carcinoma. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1194–1200.
- 10 Lappöhn RE, Burger HG, Bouma J, Bangah M, Krams M & de Bruijn HW. Inhibin as a marker for granulosa-cell tumors. *New England Journal of Medicine* 1989 **321** 790–793.
- 11 Healy DL, Burger HG, Mamers P, Jobling T, Bangah M, Quinn M *et al.* Elevated serum inhibin concentrations in postmenopausal women with ovarian tumors. *New England Journal of Medicine* 1993 **329** 1539–1542.
- 12 Cooke I, O'Brien M, Charnock FM, Groome N & Ganesan TS. Inhibin as a marker for ovarian cancer. *British Journal of Cancer* 1995 **71** 1046–1050.

- 13 Lambert-Messerlian GM, Steinhoff M, Zheng W, Canick JA, Gajewski WH, Seifer DB *et al.* Multiple immunoreactive inhibin proteins in serum from postmenopausal women with epithelial ovarian cancer. *Gynecologic Oncology* 1997 **65** 512–516.
- 14 Burger HG, Baille A, Drummond AE, Healy DL, Jobling T, Marners P *et al.* Inhibin and ovarian cancer. *Journal of Reproductive Immunology* 1998 **39** 77–87.
- 15 Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP *et al.* Measurement of dimeric inhibin B throughout the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1401–1405.
- 16 Klein NA, Illingworth PJ, Groome NP, McNeilly AS, Battaglia DE & Soules MR. Decreased inhibin B secretion in older, ovulatory women: a study of serum and follicular fluid levels of dimeric inhibin A and B in spontaneous menstrual cycles. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 2742–2745.
- 17 Ala-Fossi S-L, Mäenpää J, Bläuer M, Aine R, Tuohimaa P & Punnonen R. Inhibin A and B in peri- and postmenopause. *Maturitas* 1998 **30** 273–281.
- 18 Groome NP, Illingworth PJ, O'Brien M, Priddle J, Weaver K & McNeilly AS. Quantification of inhibin pro- α C-containing forms in human serum by a new ultrasensitive two-site enzyme-linked immunosorbent assay. *Journal of Clinical Endocrinology and Metabolism* 1995 **80** 2926–2932.
- 19 Florio P, Luisi S, Vigano P, Busacca M, Fadalti M, Genazzani AR *et al.* Healthy women and patients with endometriosis show high concentrations of inhibin A, inhibin B, and activin A in peritoneal fluid throughout the menstrual cycle. *Human Reproduction* 1998 **13** 2606–2611.
- 20 Groome NP, Illingworth PJ, O'Brien M, Pai R, Rodger FE, Mather JP *et al.* Measurement of dimeric inhibin B throughout the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 1401–1405.
- 21 Frias A, Li H, Keeney G, Podratz K & Woodruff T. Preoperative serum level of inhibin A is an independent prognostic factor for the survival of postmenopausal women with epithelial ovarian carcinoma. *Cancer* 1999 **85** 465–471.
- 22 Voutilainen R, Erämaa M & Ritvos O. Hormonally regulated inhibin gene expression in human and adult adrenals. *Journal of Clinical Endocrinology and Metabolism* 1991 **73** 1026–1030.
- 23 Roberts VJ, Barth S, El-Roeiy A & Yen SSC. Expression of inhibin/activin subunits and follistatin ribonucleic-acids and proteins in ovarian follicles and the corpus luteum during the human menstrual cycle. *Journal of Clinical Endocrinology and Metabolism* 1993 **77** 1402–1410.
- 24 Anderson RA, Groome NP & Baird T. Inhibin A and inhibin B in women with polycystic ovarian syndrome during treatment with FSH to induce mono-ovulation. *Clinical Endocrinology* 1998 **48** 577–584.
- 25 Petraglia F, Luisi S, Pautier P, Sabourin J-C, Rey R, Lhomme C *et al.* Inhibin B is the major form of inhibin/activin family secreted by granulosa cell tumors. *Journal of Clinical Endocrinology and Metabolism* 1998 **83** 1029–1032.
- 26 Robertson DM, Cahir N, Burger H, Marners P, McCloud P, Pettersson K *et al.* Combined inhibin and CA 125 assays in the detection of ovarian cancer. *Clinical Chemistry* 1999 **45** 651–658.
- 27 Hemmings R, Falcone T, Billiar R & Tulandi T. Peritoneal fluid inhibin during the menstrual cycle. *Obstetrics and Gynecology* 1992 **80** 27–29.
- 28 Billiar RB, Hemmings R, Smith P & Groome N. Identification of biologically active inhibin in the peritoneal fluid of women. *Journal of Assisted Reproduction and Genetics* 1995 **12** 55–60.
- 29 Matzuk MM, Finegold MJ, Su JE, Hsueh AJ, Bradley A *et al.* Alpha-inhibin is a tumour-suppressor gene with gonadal specificity in mice. *Nature* 1992 **360** 313–319.

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