Human Reproduction Vol.16, No.7 pp. 1359–1364, 2001

Absent biologically relevant associations between serum inhibin B concentrations and characteristics of polycystic ovary syndrome in normogonadotrophic anovulatory infertility

Joop S.E.Laven[1], Babek Imani[1], Marinus J.C.Eijkemans[2], Frank H.de Jong[3] and Bart C.J.M.Fauser[1]

[1]Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, [2]Centre for Clinical Decision Sciences, Department of Public Health and [3]Department of Internal Medicine, Erasmus University Medical Centre Rotterdam, Rotterdam, The Netherlands

[4]To whom correspondence should be addressed at: Division of Reproductive Medicine, Department of Obstetrics and Gynaecology, Erasmus University Medical Centre Rotterdam, Dr. Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.
E-mail: laven@gyna.azr.nl

BACKGROUND: Dominant follicle selection is disturbed in normogonadotrophic anovulatory infertility [World Health Organization (WHO) 2] and remaining early antral follicles are either healthy or atretic. This study was conducted to investigate whether inhibin B serum concentrations (produced by healthy small antral follicles) represent the extent of ovarian abnormalities in WHO 2 women and patients with polycystic ovarian syndrome (PCOS), constituting a subgroup of WHO 2 patients. METHODS AND RESULTS: Ultrasonographic and endocrine characteristics in 379 WHO 2 patients and 30 normo-ovulatory controls were compared. In the WHO 2 patients, the PCOS subgroup and the controls, inhibin B concentrations were similar. Inhibin B concentrations were weakly but significantly correlated with the total number of ovarian follicles ($r = 0.282$; $P < 0.001$), LH ($r = 0.347$; $P < 0.001$), and testosterone ($r = 0.269$; $P < 0.001$) but not with serum oestradiol concentrations ($r = 0.057$). Most (71%) patients with elevated inhibin B also presented with increased concentrations of LH and/or hyperandrogenaemia. In a subgroup of 190 subjects, classified as PCOS based on hyperandrogenaemia and polycystic ovaries, elevated inhibin B concentrations were found in 23% of cases. Aforementioned correlations were similar in PCOS as in WHO 2 patients. CONCLUSION: In conclusion, inhibin B serum concentrations are normal in WHO 2 and PCOS women, suggesting a normal number of healthy early antral follicles despite increased overall follicle numbers in PCOS.

Key words: androgens/gonadotrophins/inhibin B/normogonadotrophic anovulatory infertility/PCOS

Introduction

Chronic anovulation constitutes a major proportion of infertile couples (20–25%) (van Santbrink *et al.*, 1997). According to the World Health Organization (WHO) (Rowe *et al*., 2000), anovulatory patients are classified on the basis of two endocrine parameters, the concentrations of endogenous gonadotrophins and oestrogens. Approximately 80% of patients suffering from chronic anovulation present with serum FSH concentrations within the normal range in combination with some endogenous oestrogen activity. These women are classified as normo-gonadotrophic normo-oestrogenic anovulatory infertility, more commonly referred to as WHO 2 (Rowe *et al.*, 2000). Since aetiological factors underlying this condition may vary from one patient to another, WHO 2 anovulatory women constitute a notoriously heterogeneous population.

The polycystic ovarian syndrome (PCOS), characterized by chronic anovulation, hyperandrogenaemia and sometimes hyperinsulinaemia (Dunaif, 1999), is the most common cause of normogonadotrophic normo-oestrogenic anovulatory infertility. Certainly, PCOS is part of the WHO 2 group, with a variable reported incidence based on differences in inclusion criteria used (van Santbrink, 1997). A previous National Institute of Health (NIH) consensus workshop concluded, but by no means unanimously, that hyperandrogenaemic chronic anovulation should be considered the hallmark criterion for the diagnosis of PCOS (Dunaif *et al.*, 1992). More recently, it was reported (and agreed by the last NIH consensus workshop) that the occurrence of polycystic ovaries should be added (Dewailly, 2000).

Inhibin is a dimeric non-steroidal glycoprotein hormone that selectively inhibits FSH production and/or release from the pituitary. It consists of two partially homologous sub-units,

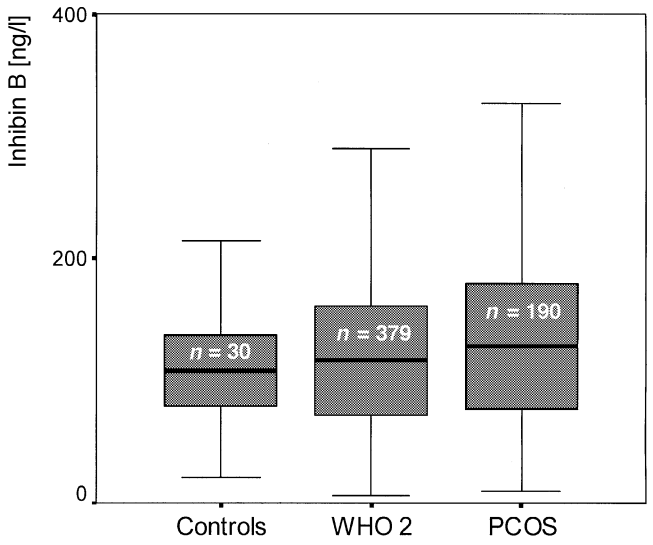

Figure 1. Box and whisker plots indicating median inhibin B serum concentrations (solid bars) with 25th and 75th quartiles (lower and upper borders of boxes) and ranges (whiskers) in normogonadotrophic oligoamenorrhoeic infertile [World Health Organization (WHO 2)] and in a subgroup of polycystic ovarian syndrome (PCOS) women during the early follicular phase in regularly cycling controls.

Table I. Clinical, endocrine and ultrasound characteristics (median and range) in 379 patients with normogonadotrophic anovulatory infertility [World Health Organization (WHO 2)] and separately in the subgroup of 190 polycystic ovarian syndrome (PCOS) patients

	WHO 2 (n = 379)	PCOSa (n = 190)
Clinical		
Age (years)	28 (16.1–41.3)	27 (16.1–39.7)
BMI (kg/m^2)	26 (13.6 – 52.3)	29 (17.3–52.3)
% Amenorrhoea	25	29
Cycle length (days)	60 (35–199)	75 (35–199)
Endocrine		
LH (IU/l)	6.5 (0.2–33.3)	7.7 (1.0–24.4)
FSH (IU/l)	4.6 (1.0–10.0)	4.5 (1.0–9.9)
LH/FSH ratio	1.6 (0.1–6.4)	1.8 (0.2–6.4)
Oestradiol (pmol/l)	221 (29–1868)	228 (75–1173)
Inhibin B (ng/l)	123 (6–630)	130 (10–621)
Testosterone (nmol/l)	2.3 (0.1–6.7)	2.7 (0.9–6.7)
Androstenedione (nmol/l)	13.2 (0.8–56.9)	14.9(3.0–56.9)
DHEA-S (μmol/l)	6.8 (0.1–20.0)	7.2 (0.7–20.0)
Free androgen index	4.9 (0.2–42.7)	7.7 (4.5–42.7)
SHBG (nmol/l)	46.1 (0.1–289)	32.6 (10.9–95)
Fasting glucose/insulin	0.36 (0.1–3.9)	0.30 (0.5–4.0)
Ultrasound		
% Polycystic ovaries[b]	67	81

[a]PCOS based on hyperandrogenaemia [free androgen index (FAI) >4.5] and polycystic ovaries (mean ovarian volume > 10.8 m).
[b]Defined as increased mean ovarian volume (>10.8 ml) and an increased number of follicles per ovary (>10).
BMI = body mass index; DHEA-S = dehydroepiandrosterone sulphate; SHBG = sex hormone binding globulin.

α combined with either βA (inhibin A) or βB (inhibin B) (Robertson *et al.*, 1997). Recently, it was reported that inhibin A serum concentrations are high around ovulation and during the mid-luteal phase of the normal menstrual cycle. In contrast, inhibin B seems to be the predominant form secreted by developing small antral follicles during the early follicular phase (Groome *et al.*, 1996). Recent reports on serum and follicular fluid inhibins in PCOS are conflicting. According to some authors, concentrations of total immunoreactive inhibins (Mizunuma *et al.*, 1994), α inhibin (Pigny *et al.*, 1997), or inhibin B (Lambert-Messerlian *et al.*, 1994; Anderson *et al.*, 1998; Lockwood *et al.*, 1998) are raised. Others found normal concentrations of total immunoreactive inhibin (Buckler *et al.*, 1988; Pache *et al.* 1992) or inhibin B (Magoffin and Jakimiuk, 1998).

Since inhibin B selectively inhibits FSH, high inhibin B could be held responsible for an elevated LH/FSH ratio characteristic for some patients (Magoffin and Jakimiuk, 1998). Moreover, inhibin may directly stimulate theca cell androgen biosynthesis (Hillier *et al.*, 1991). Inhibin B concentrations might also represent the extent of ovarian dysfunction in these patients, since an increased number of healthy follicles (Hughesdon, 1982; Pache *et al.*, 1991a) may result in increased serum concentrations. The present cross-sectional study in a large cohort of normogonadotrophic anovulatory infertile patients was conducted to investigate whether inhibin B concentrations were correlated with endocrine and ultrasound findings in WHO 2 and PCOS patients.

Materials and methods

Patients

The local Medical Ethics Review Committee approved this study and informed consent was obtained from all participants. Three hundred and seventy-nine patients attending our fertility clinic between 1994 and 1999 with: (i) infertility, (ii) oligomenorrhea (interval between periods >35 days) or amenorrhea (absence of vaginal bleeding for at least 6 months), (iii) serum FSH concentrations within normal limits (1–10 IU/l) (van Santbrink *et al.*, 1995; Schipper *et al.*, 1998), (iv) positive withdrawal bleeding after progestagen administration in case of amenorrhea, and (v) between 20 and 40 years of age were included in the present study. Standardized initial screening (clinical, transvaginal ultrasound and fasting blood withdrawal) was performed on a random day between 9 and 11 a.m. as previously described (van Santbrink *et al.*, 1997). A PCOS subgroup of patients exhibited hyperandrogenaemia and an increased ovarian volume. Hyperandrogenaemia was defined as an elevated (>4.5) free androgen index [testosterone×100/sex hormone binding globulin (SHBG)], whereas the ovarian volume was considered increased above 10.8 ml (van Santbrink *et al.*, 1997).

For sonographic imaging, a 6.5 MHz vaginal transducer (model EUB-415; Hitachi Medical Corporation, Tokyo, Japan) was used. The ovaries were localized and scanned as described previously (Pache *et al.*, 1991b). Ovarian volume, stroma echogenicity (arbitrarily scored from 1 to 3 per ovary) as well as the mean follicle number were assessed as described earlier (van Santbrink *et al.*, 1997).

The control group consisted of 30 healthy volunteers selected by advertisement and paid for participation as previously published (Schipper *et al.*, 1998). Inclusion criteria were a regular menstrual cycle (26–30 days), 20–35 years of age, normal body mass index (BMI 18–25 kg/m^2) and no previous use of medication or oral contraceptives during at least 3 months prior to the study. Transvaginal ultrasound and blood sampling were performed during the early follicular phase (cycle day 3, 4 or 5).

Table II. Correlations between inhibin B serum concentrations and clinical, endocrine and ultrasound parameters in 379 patients with normogonadotrophic anovulatory infertility (WHO 2), and separately in the subgroup of 190 PCOS patients

	WHO 2 (n = 379) Correlation coefficient and P value[a,b]		PCOS (n = 190)	
Age (years)	–0.131[a]	(0.011)[a]	–0.004	(0.951)
BMI (kg/m^2)	–0.294	(<0.001)	–0.334	(<0.001)
Cycle length (days)	–0.024	(NS)	–0.131	(0.071)
LH (IU/l)	0.347	(<0.001)	0.405	(0.001)
FSH (IU/l)	0.217	(<0.001)	0.204	(0.005)
LH/FSH ratio	0.203	(0.024)	0.236	(0.001)
Oestradiol (pmol/l)	0.057	(NS)	0.123	(0.091)
Testosterone (nmol/l)	0.269	(<0.001)	0.247	(0.001)
Androstenedione (nmol/l)	0.196	(<0.001)	0.169	(0.020)
DHEAS (µmol/l)	0.026	(0.609)	0.008	(0.915)
FAI	–0.003	(NS)	–0.150	(0.039)
SHBG (nmol/l)	0.179	(<0.001)	0.331	(<0.001)
Fasting glucose/insulin	0.129	(0.013)	0.152	(0.039)
Total ovarian volume (ml)	0.216	(<0.001)	0.148	(0.042)
Mean follicle number	0.282	(<0.001)	0.148	(0.043)

[a]Spearman's correlation coefficient.
[b]P value from bivariate analysis.

Hormone assays

Blood samples were obtained by venepuncture and processed within 2 h after withdrawal. Serum was stored at –20°C and assayed for LH, FSH, androstenedione, testosterone, inhibin B, oestradiol and progesterone. Serum LH and FSH concentrations were measured by immunofluorometric assay (Amerlite, Ortho-Clinical Diagnostics, Amersham, UK), while serum oestradiol, progesterone, testosterone, androstenedione and SHBG concentrations were measured by radioimmunoassay (RIA) provided by Diagnostic Products Corp. (DPC, Los Angeles, CA, USA), as described previously (Imani *et al.*, 2000). Intra- and inter-assay coefficients of variation were <5 and 15% for LH, <3 and 8% for FSH, <8 and 11% for androstenedione, <3 and 5% for testosterone, <5 and 7% for oestradiol, <16 and 17% for progesterone, and <4 and 5% for SHBG respectively. Dimeric inhibin B concentrations were assessed using an immuno-enzymometric assay obtained from Serotec (Oxford, Oxon, UK), as described previously (Schipper *et al.*, 1998). The detection limit of the assay, defined as the amount of inhibin equivalent with the signal of the blank +3 SDs of this signal, was 3.4 ng/l. Intra- and inter-assay coefficients of variation for inhibin B were <9 and 15% respectively.

Data analysis

Data are presented as median and range. Due to the non-parametric distribution, groups were compared using the Mann–Whitney rank-sum test. Non-parametric cross correlations and analysis of covariance was performed using a commercially available software package (Statistics Package for Social Sciences, Chicago, IL, USA). A P value < 0.05 was considered to indicate statistical significance.

Results

Normo-ovulatory controls (n = 30) presented with a median age of 29 years (range 20–35 years), median cycle duration of 28 days (range 25–32 days) and median BMI of 21.4 kg/m^2 (range 18.9–24.2 kg/m^2). The upper limit of normal inhibin B was 213 ng/l (95th percentile) and the lower limit (5th percentile) was 22 ng/l (Figure 1). Medians of inhibin B on cycle day 3, 4 and 5 were 103, 130 and 135 pg/l respectively. These values were not statistically different. For further comparisons with WHO 2 patients and PCOS patients, day 3 concentrations of inhibin B in controls were used.

Clinical, endocrine and ultrasound parameters of WHO 2 and PCOS subgroup are depicted in Table I. The distribution of individual inhibin B serum concentrations in WHO 2, PCOS and controls is depicted in Figure 1. Correlations between serum inhibin B concentrations and clinical, endocrine and ultrasound parameters in the WHO 2 and PCOS individuals are depicted in Table II as well as in Figures 2 and 3. Covariance analysis revealed that the interrelationship between inhibin B, FSH, age, and mean follicle number was predominantly determined by the serum FSH concentration. Similarly, co-variance analysis revealed that the interrelationship between inhibin B, LH, testosterone, and androstenedione was mainly determined through the serum LH concentration (data not shown).

In WHO 2 patients polycystic ovaries on ultrasound examination (defined as a mean ovarian volume ⩾10.8 ml and/or a mean follicle number per ovary ⩾10) (van Santbrink *et al.*, 1997) was observed in 67% of all patients. Elevated LH and/or testosterone was found in 54%, whereas an elevated inhibin B concentration was found in 15% of all WHO 2 patients. A total of 71% of WHO 2 individuals with elevated inhibin B also presented with increased concentrations of LH and/or increased testosterone. Similarly, 43% of all WHO 2 patients showed elevated serum concentrations of LH and/or testosterone in combination with polycystic ovaries on ultrasound. In PCOS patients, elevated serum LH concentrations were found in 58% of all individuals whereas elevated serum inhibin B concentrations were found in only 23% of all cases. An elevated testosterone plasma concentration was observed in 35% of PCOS patients.

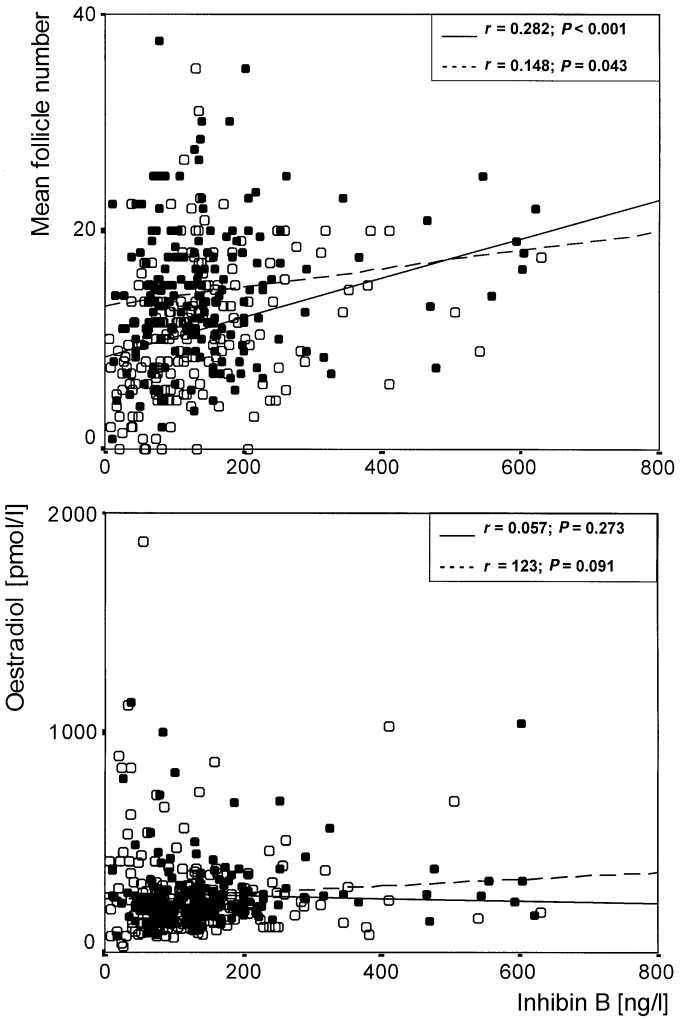

Figure 2. Correlations between inhibin B serum concentrations and mean follicle number (upper panel) and serum oestradiol concentrations (lower panel) in 379 normogonadotrophic oligoamenorrhoeic infertile patients (open and closed dots and continuous line) and in a subgroup of 190 PCOS (closed dots and dotted line) women. Spearman's correlation coefficients and corresponding *P* values are depicted.

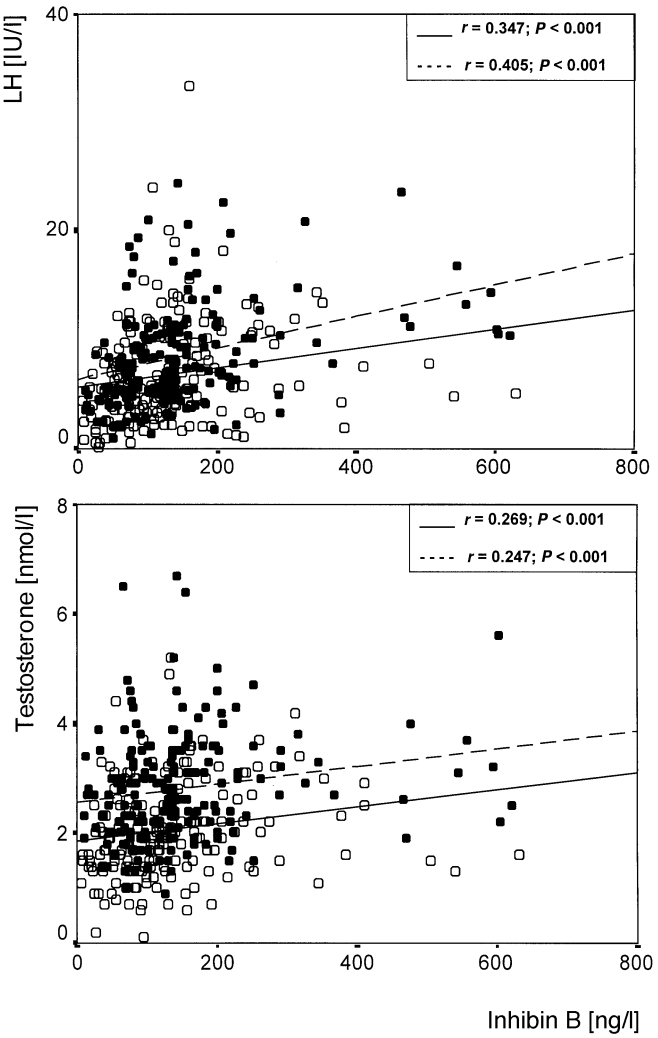

Figure 3. Correlations between inhibin B serum concentrations and serum LH (upper panel) and serum Testosterone concentrations (lower panel) in 379 normogonadotrophic oligoamenorrhoeic infertile patients (open and closed dots and continuous line) and in a subgroup of 190 PCOS (closed dots and dotted line) women. Spearman's correlation coefficients and corresponding *P* values are depicted.

Discussion

The data presented here in 379 normogonadotrophic oligomenorrheic patients indicate that overall inhibin B serum concentrations are elevated neither in the total group of WHO 2 women nor in the subgroup of PCOS patients. 'High' inhibin B concentrations reported in previous studies (Anderson *et al.*, 1998; Lockwood *et al.*, 1998) were well within the normal range of regularly cycling women in the current study. This discrepancy in absolute values might be due to modifications in the assay that may affect absolute concentrations (N.P.Groome, personal communication). In one study (Lockwood *et al.*, 1998), inhibin B concentrations were assessed on day 5 of the menstrual cycle in contrast to the current study, in which they were measured on day 3. However, inhibin B shows only modest changes at the beginning of the cycle (Groome *et al.*, 1996). In the current series, inhibin B serum concentrations were similar on day 3 compared to days 4 or 5 (data not shown). Moreover, differences in inhibin B concentrations in control subjects (Anderson *et al.*, 1998) might be due to the smaller number of regularly cycling women (n = 10 and 5 respectively) compared with the current study (n = 30). Finally, inhibin B is secreted in a pulsatile fashion (Lockwood *et al.*, 1998) which may affect the accuracy of single blood withdrawal. Besides, one study (Lockwood *et al.*, 1998) included only clomiphene-resistant women, which might represent a different subset of patients.

Dominant follicle selection is disturbed in polycystic ovaries, resulting in an increased number of follicles per ovary and presumably a variable number of healthy early antral follicles (Fauser, 1994). Because inhibin B is produced predominantly by healthy small follicles it has been postulated that inhibin B concentrations are increased in at least some PCOS patients. Elevated concentrations of inhibin B could be established in two preliminary reports (Anderson *et al.*, 1998; Lockwood

et al., 1998) in 9 and 10 PCOS patients respectively. In contrast, in the present study, inhibin B concentrations in PCOS patients were similar compared with WHO 2 and control patients.

It has also been postulated that most of the inhibin B produced in the ovary during the follicular phase originates from the dominant follicle due to the observed major difference in the amount produced by large antral follicles (with androstenedione/oestradiol ratios ≤ 4) compared with remaining cohort follicles with androstenedione/oestradiol ratios >4 (Magoffin and Jakimiuk, 1998). Follicular fluid oestradiol, androstenedione and total immunoreactive inhibin concentrations as well as androstenedione/oestradiol ratios were similar in PCOS patients compared to controls (Pache *et al.*, 1992). More recently, inhibin B concentrations in these 4–8 mm follicles from polycystic ovaries were also found to be similar to size-matched normal follicles (Magoffin and Jakimiuk, 1998). Increased inhibin secretion by polycystic ovaries could augment LH-stimulated androgen production by theca cells (Hillier *et al.*, 1991). Recent observations in PCOS patients supporting this concept include elevated α inhibin concentrations, which were correlated with increased circulating concentrations of androstenedione (Pigny *et al.*, 1997). It was not possible to demonstrate a consistent and strong correlation between inhibin B and LH, testosterone or its precursors in the current study. It therefore appears that alterations in inhibin B concentrations do not play an important local role *in vivo* in LH-induced androgen production in these patients.

If serum inhibin B concentrations represent the extent of ovarian dysfunction in PCOS, one would expect a good correlation between inhibin B and endocrine hallmarks associated with ovarian abnormalities in these patients, such as elevated serum LH or androgen concentrations. Although ultrasound parameters are weakly correlated with inhibin B, serum oestradiol concentrations were not. In fact, serum oestradiol concentrations were fairly constant at different inhibin B concentrations. The observed lack of a strong, consistent relationship between inhibin B and endocrine markers of PCOS may suggest that inhibin B does not represent the magnitude of ovarian dysfunction in these patients. These results are consistent with recent observations from our group indicating that inhibin B serum concentrations do not predict ovarian responsiveness to ovulation induction (Imani *et al.*, 2000).

It has been postulated that if inhibin is prematurely produced in excessive amounts relative to the developmental stage of the follicle, FSH secretion by the pituitary might become suppressed and concomitantly follicle development could be retarded (Magoffin and Jakimiuk, 1998). If this is true, inhibin B concentrations should be negatively correlated with serum FSH. Based on the observed weak positive correlation between serum inhibin B and FSH concentrations, the current data do not support this concept. The present study supports the contention that FSH is the primary drive of inhibin B synthesis, in line with recent observations during pubertal development (Crofton *et al.*, 1997). Since inhibin B concentrations in PCOS patients (produced by small arrested follicles) are similar to control concentrations, one might speculate that inhibin B exerts limited negative feedback on pituitary FSH production at the beginning of the cycle. Moreover, intra-follicular inhibin B concentrations in PCOS do not significantly decrease with increasing follicle diameters also indicating that FSH is the primary drive of the feedback (Lambert-Messerlian *et al.*, 1997).

In conclusion, inhibin B serum concentrations are normal in most normogonadotrophic anovulatory infertile women, including PCOS, suggesting a normal number of healthy early antral follicles despite increased overall follicle numbers in PCOS. Serum inhibin B assays cannot be recommended for routine screening in normogonadotrophic anovulatory infertility.

References

Anderson, R.A., Groome, N.P. and Baird, D.T. (1998) Inhibin A and inhibin B in women with polycystic ovarian syndrome during treatment with FSH to induce mono-ovulation. *Clin. Endocrinol. (Oxf)*, **48**, 577–584.

Buckler, H.M., McLachlan, R.I., MacLachlan, V.B. *et al.* (1988) Serum inhibin levels in polycystic ovary syndrome: basal levels and response to luteinizing hormone-releasing hormone agonist and exogenous gonadotropin administration. *J. Clin. Endocrinol. Metab*, **66**, 798–803.

Crofton, P.M., Illingworth, P.J., Groome, N.P. *et al. (*1997) Changes in dimeric inhibin A and B during normal early puberty in boys and girls. *Clin. Endocrinol. (Oxf)*, **46**, 109–114.

Dewailly, D. (2000) [Polycystic ovary syndrome] Le syndrome des ovaires polymicrokystiques. *J. Gynecol. Obstet. Biol. Reprod.*, *(Paris)*, **29**, 298–301.

Dunaif, A. (1999) Insulin action in the polycystic ovary syndrome. *Endocrinol. Metab Clin. North Am.*, **28**, 341–359.

Dunaif, A., Givens, J.R., Haseltine, F. *et al.* (1992) In Dunaif,A., Givens, J.R., Haseltine, F. *et al* (eds), *The Polycystic Ovary Syndrome.* Blackwell Scientific, Cambridge.

Fauser, B.C. (1994) Observations in favor of normal early follicle development and disturbed dominant follicle selection in polycystic ovary syndrome. *Gynecol. Endocrinol.*, **8**, 75–82.

Groome, N.P., Illingworth, P.J., O'Brien, M. *et al.* (1996) Measurement of dimeric inhibin B throughout the human menstrual cycle. *J. Clin. Endocrinol. Metab.*, **81**, 1401–1405.

Hillier, S.G., Yong, E.L., Illingworth, P.J. *et al.* (1991) Effect of recombinant inhibin on androgen synthesis in cultured human thecal cells [published erratum appears in *Mol. Cell. Endocrinol.*, 1991, **79**, 177]. *Mol. Cell. Endocrinol.*, **75**, R1–R6.

Hughesdon, P.E. (1982) Morphology and morphogenesis of the Stein–Leventhal ovary and of so-called 'hyperthecosis'. *Obstet. Gynecol. Surv.*, **37**, 59–77.

Imani, B., Eijkemans, M.J., de Jong, F.H. *et al.* (2000) Free androgen index and leptin are the most prominent endocrine predictors of ovarian response during clomiphene citrate induction of ovulation in normogonadotropic oligoamenorrheic infertility. *J. Clin. Endocrinol. Metab.*, **85**, 676–682.

Lambert-Messerlian, G.M., Hall, J.E., Sluss, P.M. *et al.* (1994) Relatively low levels of dimeric inhibin circulate in men and women with polycystic ovarian syndrome using a specific two-site enzyme-linked immunosorbent assay. *J. Clin. Endocrinol. Metab.*, **79**, 45–50.

Lambert-Messerlian, G., Taylor, A., Leykin, L. *et al.* (1997) Characterization of intrafollicular steroid hormones, inhibin, and follistatin in women with and without polycystic ovarian syndrome following gonadotropin hyperstimulation. *Biol. Reprod.*, **57**, 1211–1216.

Lockwood, G.M., Muttukrishna, S., Groome, N.P. *et al.* (1998) Mid-follicular phase pulses of inhibin B are absent in polycystic ovarian syndrome and are initiated by successful laparoscopic ovarian diathermy: a possible mechanism regulating emergence of the dominant follicle. *J. Clin. Endocrinol. Metab.*, **83**, 1730–1735.

Magoffin, D.A. and Jakimiuk, A.J. (1998) Inhibin A, inhibin B and activin A concentrations in follicular fluid from women with polycystic ovary syndrome. *Hum. Reprod.*, **13**, 2693–2698.

Mizunuma, H., Andoh, K., Obara, M. *et al.* (1994) Serum immunoreactive inhibin levels in polycystic ovarian disease (PCOD) and hypogonadotropic amenorrhea. *Endocr. J.*, **41**, 409–414.

Pache, T.D., Chadha, S., Gooren, L.J. *et al.* (1991a) Ovarian morphology in

long-term androgen-treated female to male transsexuals. A human model for the study of polycystic ovarian syndrome? *Histopathology*, **19**, 445–452.

Pache, T.D., Hop, W.C., Wladimiroff, J.W. *et al.* (1991b) Transvaginal sonography and abnormal ovarian appearance in menstrual cycle disturbances. *Ultrasound Med. Biol.*, **17**, 589–593.

Pache, T.D., Hop, W.C., de Jong, F.H. *et al.* (1992) 17 beta-Oestradiol, androstenedione and inhibin levels in fluid from individual follicles of normal and polycystic ovaries, and in ovaries from androgen treated female to male transsexuals. *Clin. Endocrinol. (Oxf)*, **36**, 565–571.

Pigny, P., Desailloud, R., Cortet-Rudelli, C. *et al.* (1997) Serum alpha-inhibin levels in polycystic ovary syndrome: relationship to the serum androstenedione level. *J. Clin. Endocrinol. Metab.*, **82**, 1939–1943.

Robertson, D.M., Cahir, N., Findlay, J.K. *et al.* (1997) The biological and immunological characterization of inhibin A and B forms in human follicular fluid and plasma. *J. Clin. Endocrinol. Metab.*, **82**, 889–896.

Rowe, P.J., Comhaire, F.H., Hargreave, T.B. *et al.* (2000) Female partner. In Rowe, P.J., Comhaire, F.H., Hargreave, T.B. *et al.* (eds), *WHO Manual for the Standardized Investigation and Diagnosis of the Infertile Couple.* Press Syndicate of the University of Cambridge, Cambridge, pp. 40–67.

Schipper,I., de Jong,F.H. and Fauser,B.C. (1998) Lack of correlation between maximum early follicular phase serum follicle stimulating hormone concentrations and menstrual cycle characteristics in women under the age of 35 years. *Hum. Reprod.*, **13**, 1442–1448.

van Santbrink, E.J., Hop, W.C., van Dessel, T.J. *et al.* (1995) Decremental follicle-stimulating hormone and dominant follicle development during the normal menstrual cycle. *Fertil. Steril.*, **64**, 37–43.

van Santbrink, E.J., Hop, W.C. and Fauser, B.C. (1997) Classification of normogonadotropic infertility: polycystic ovaries diagnosed by ultrasound versus endocrine characteristics of polycystic ovary syndrome. *Fertil. Steril.*, **67**, 452–458.

Received on December 7, 2000; accepted on March 19, 2001