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## ARTICLE

# Serum inhibin A concentration in women with polycystic ovarian syndrome and the correlation to ethnicity, androgens and insulin resistance

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**Abstract** A prospective case-series in an academic hospital clinic was performed to determine whether there is a relationship between polycystic ovarian syndrome (PCOS) and ethnicity. Also, serum inhibin A concentrations were compared between PCOS and normal-ovulatory women. The possibility of a correlation between inhibin A, androgens and insulin resistance in PCOS women was evaluated. Serum inhibin A concentrations were measured in anovulatory PCOS patients ( $n = 32$ ) and in control women of reproductive age ( $n = 16$ ). Statistical analysis was performed using the Mann–Whitney  $U$ -test. Serum concentrations of inhibin A, follicle-stimulating hormone, LH, prolactin, thyroid-stimulating hormone, fasting glucose, insulin, testosterone, 17-hydroxyprogesterone (17-OHP) and dehydroepiandrosterone sulphate (DHEAS) were measured. Inhibin A concentrations were significantly lower ( $4.5 \pm 4.8$  pg/ml) when compared with the control group ( $13.2 \pm 14.4$  pg/ml;  $P = 0.003$ ) and were not significantly different between Hispanic and Caucasian women diagnosed with PCOS. There was no correlation between inhibin A concentrations and insulin, testosterone, free testosterone, 17-OHP, or DHEAS concentrations. In PCOS women, inhibin A concentrations are similar between Hispanic and Caucasian women; however, women with PCOS, regardless of ethnicity, have a lower inhibin A concentration compared with normal-ovulatory women. No correlation was observed between inhibin A androgens and insulin resistance in women diagnosed with PCOS. [RBMO Online](#)

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**KEYWORDS:** Caucasian, ethnicity, Hispanic, inhibin A, polycystic ovarian syndrome

## Introduction

Polycystic ovarian syndrome (PCOS) is characterized by a state of anovulation that may persist for any length of time. PCOS is considered one of the most common endocrine disorders impacting women of reproductive age. As a syndrome, clinical and endocrinological features may manifest as menstrual irregularity, androgen excess, insulin resistance and infertility.

At the European Society for Reproduction and Embryology/American Society for Reproductive Medicine (ESHRE/ASRM) joint meeting, a panel concluded that a diagnosis of PCOS could be attributed to patients having at least two of the following three criteria after ruling out other aetiologies (congenital adrenal hyperplasia, androgen-secreting tumours and Cushing's syndrome; Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2004): (i) oligomenorrhoea; (ii) hyperandrogenism; and (iii) ultrasound findings of polycystic ovaries, defined as multiple follicles <10 mm in the classic 'pearl necklace' pattern beneath the ovarian capsule.

With the isolation of inhibin, it was found that ovarian inhibin exerts negative feedback on pituitary gonadotrophin production, preferentially affecting follicle-stimulating hormone (FSH) secretion. While its full significance has yet to be determined, inhibin has been shown to impact basal secretion of both FSH and LH, as well as gonadotrophin-releasing hormone (GnRH)-stimulated FSH (Burger, 1992).

Studies using different animal models have been previously conducted. Rivier et al. (1991a,b) elucidated the impact of inhibin on gonadotrophin when recombinant human inhibin A was injected into female rats. This study demonstrated that while preventing the pro-estrous evening FSH surge, it did not inhibit ovulation. When FSH pulse parameters were evaluated, Rivier et al. (1991b) found that recombinant human inhibin impacted pulse frequency, amplitude and peak concentrations in intact and ovariectomized rats, yet LH secretion did not appear to be affected. This data suggests that inhibin alters mostly the pituitary sensitivity to GnRH, not the hypothalamic GnRH release. Consistent with this, Molskness et al. (1996) found that FSH secretion rapidly diminished when female rhesus monkeys with regular menstrual cycles were injected with inhibin thus identifying how inhibin influences the ovarian cycle in primates. Lastly, in an effort to better define the role of inhibin, Stouffer et al. (1994) infused recombinant human inhibin A into rhesus monkeys and found that while progesterone, oestradiol and LH concentrations and the length of the luteal phase were not significantly affected, FSH concentrations declining to 26% of pretreatment concentrations. Ultimately, while its full significance has yet to be determined, inhibin has been shown to impact basal secretion of FSH and LH, as well as GnRH-stimulated FSH (Burger, 1992).

Abnormalities of inhibin secretion have long been postulated in the pathogenesis of PCOS. Lord and Wilkin (2002) reported that inhibin B secretion by the ovary in PCOS women suppresses pituitary secretion of FSH. Significantly elevated serum concentrations of inhibin B have been observed in PCOS women (Lockwood, 2000), suggesting that it may be responsible for the reversible FSH:LH ratio and the in-

creased sensitivity of the polycystic ovary to exogenous FSH. Further, a different study (Lockwood et al., 1998) noted that the raised inhibin B concentrations found in PCOS results in suppression of FSH and failure of the dominant follicle selection. Inhibin A and inhibin B were elevated in women with PCOS during treatment with FSH to induce mono-ovulation (Anderson et al., 1998). Tsigkou et al. (2008) was unable to find a correlation between total inhibin and the individual concentrations of inhibin A or inhibin B in women with PCOS. This data suggest that women suffering from PCOS may have an impaired processing of -inhibin precursor proteins.

While it is known that inhibin increases androgen production and androgen in turn stimulates inhibin secretion, the mechanism is not well defined. The potential exists for the development of a cycle within the ovary that would serve to inhibit follicle development, which may explain the relatively low serum concentrations of FSH compared with LH in anovulatory women with PCOS.

The role of inhibin A during menstrual cycle has been evaluated. Inhibin A is secreted mainly from the follicular granulosa cells, predominantly by the dominant follicle. Inhibin A concentrations rise in the late follicular phase to reach a peak in the midluteal phase which may contribute to the suppression of FSH to nadir concentrations during the luteal phase and to the changes at the luteal follicular transition (Groome and O'Brien, 1993; Muttukrishna et al., 1995).

An assay developed at the Monash Institute was employed (Burger et al., 1996) but it did not distinguish between the varying molecular forms of inhibin, some of which are biologically inactive. More recently, a specific assay for the quantitative determination of dimeric inhibin A in human serum and plasma has been developed (Groome and O'Brien, 1993).

Inhibin A, a 31 kD disulphide-linked heterodimeric protein hormone, was measured in serum during normal-ovulatory menstrual cycles. The test method is based on the two subunit-specific antibodies enzyme-linked immunosorbent assay (ELISA) method (Groome and O'Brien, 1993; Muttukrishna et al., 1995). The stability of inhibin A in serum samples over time has been demonstrated previously (Segal et al., 2008).

Inhibin A concentrations have been previously measured in granulosa-cell RNA obtained by transvaginally aspirating follicles, suggesting a role in the pathophysiology of PCOS. Fujiwara et al. (2001) found that alpha subunit mRNA concentrations were 16-fold lower in PCOS patients, indicating that insufficient inhibin may be associated with follicular arrest in PCOS follicles. Magoffin and Jakimiuk (1998) reported similar findings and Tanabe et al. (1990) found that inhibin A concentrations in follicular fluid of PCOS patients were lower than concentrations observed in normal cohort follicles.

There are conflicting reports regarding inhibin A concentrations in serum of PCOS women. Lambert-Messerlian et al. (1994) reported that serum dimeric inhibin concentrations in PCOS patients were similar to normal follicular phase. In contrast, Pigny et al. (2000) evaluated serum inhibin A concentrations in PCOS patients in the early follicular phase and found them lower than those seen in the control group. The physiology and pathophysiology of inhibin was reviewed

(Welt et al., 2002) and the role of inhibin in PCOS remains unclear (Kumanov et al., 2005).

Whether ethnicity can be a predictor of PCOS has not yet been established. A higher prevalence of PCOS has been reported in Hispanic women compared with Caucasian women (Azziz et al., 2004) and Mexican-American women with PCOS exhibit greater insulin resistance than their non-Hispanic, Caucasian counterparts (Goodarzi et al., 2005; Kauffman et al., 2002).

Conversely, Legro et al. (2006) looked at PCOS women and was unable to find significant differences in baseline biochemical measures among different ethnicities. The study looked at testosterone, sex hormone-binding globulin, insulin, proinsulin and glucose. The free-androgen index, defined as total testosterone/sex hormone-binding globulin, was also calculated.

This study was designed to determine the existence of: (i) a difference in inhibin A concentrations in PCOS women compared with normal-ovulatory women; (ii) a correlation between serum inhibin A concentrations in women with PCOS and ethnicity; and (iii) a correlation in PCOS women between inhibin A androgens and insulin resistance.

## Materials and methods

### Patients

This prospective case–control study measured inhibin A in women who presented to the outpatient Reproductive Medicine Clinic of the Department of Obstetrics and Gynecology, an academic teaching clinic between 2002 and 2004, for evaluation of oligoovulation or infertility ( $n = 42$ ). Menstrual bleeding either occurred spontaneously or was induced with medroxyprogesterone acetate supplied by Greenstone (USA) 10 mg for 10 days. Blood was then drawn on day 3 of the menstrual cycle after the patient had fasted overnight.

Diagnosis of PCOS was based upon the defining criteria set forth by the PCOS Consensus Group in Rotterdam (Rotterdam ESHRE/ASRM Sponsored PCOS Consensus Workshop Group, 2004): severe amenorrhoea or chronic anovulation; hyperandrogenism (either clinically or biochemically determined); and ultrasound features of polycystic ovaries. The exclusion criteria in the PCOS patients were Cushing's syndrome, late-onset congenital hyperplasia and diabetes. In the control group, women with an irregular menstrual cycle or comorbid medical conditions were excluded.

The control group comprised women with regular menstrual cycles of reproductive age who presented to the outpatient Obstetrics and Gynecology Clinic for a routine gynecological examination during the follicular phase of the menstrual cycle ( $n = 16$ ).

### Data collection

In the PCOS group, each patient's age, height, weight and ethnicity were recorded, as well as the results of the pelvic ultrasound. Blood was drawn in the morning after fasting from midnight the day prior and inhibin A, FSH, LH, prolactin, thyroid-stimulating hormone, fasting glucose, insulin, testosterone, 17-hydroxyprogesterone (17-OHP) and dehy-

droepiandrosterone sulphate (DHEAS) concentrations were measured. The assays were performed using Siemens reagents using Centaur for Chemiluminescence system (Siemens Healthcare Diagnostics, USA). Serum collected for inhibin A was frozen at  $-20^{\circ}\text{C}$  within 2 h of drawing and stored until assayed.

In the control group, fasting blood samples were drawn and frozen. Body mass index was calculated and insulin resistance was noted if fasting insulin concentration was  $>17\ \mu\text{IU/ml}$  (upper normal concentration in the study centre's laboratory and fasting glucose to insulin ratio  $<4.5$ ).

### Inhibin A assay

Inhibin A heterodimer was measured in serum using the ultrasensitive Oxford Bioinnovation (UK) kit based on the two subunit-specific antibodies ELISA method, which have been previously described and validated (Groome and O'Brien, 1993; Muttukrishna et al., 1995). The assay is a classical ELISA assay using a capture antibody against the  $\beta_A$  subunit and a second monoclonal antibody specific for the  $\alpha$  subunit coupled to alkaline phosphatase for the detection step. The kit has a sensitivity of  $1.0\ \text{pg/ml}$  and is specific to inhibin A, with minimal cross-reactivity with the Pro(C) subunit, inhibin B or activins. Inter- and intra-plate variations are  $<10\%$ .

### Data analysis

Collected data presented as mean  $\pm$  SD were compared and analysed using the Mann–Whitney  $U$  and Fisher test. When the  $P$ -value was  $<0.05$ , the differences were considered statistically significant.

## Results

Hormone concentrations measured in the PCOS group are presented in (Table 1). When evaluating the data between the PCOS group taken as a whole and the control group, inhibin A concentrations in PCOS patients were significantly lower ( $4.5 \pm 4.8\ \text{pg/ml}$ ) compared with the control group ( $13.2 \pm 14.4\ \text{pg/ml}$ ;  $P = 0.003$ ; Figure 1).

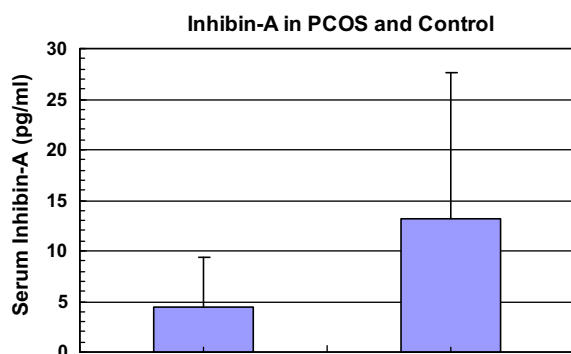
The PCOS group included 10 Caucasians and 32 Hispanics and no statistically significant differences were identified between the ethnic groups in age, body mass index (BMI), FSH, LH, prolactin, thyroid-stimulating hormone, inhibin A, glucose, insulin, testosterone, free testosterone, 17-OHP and DHEAS (Table 2). Due to the predominance of Hispanic women in the clinic, the number of patients in each group was not equal.

Inhibin A concentrations in Caucasian women with PCOS were not significantly higher compared with Hispanic women with PCOS ( $4.5 \pm 4.9\ \text{pg/ml}$  versus  $5.1 \pm 6.3\ \text{pg/ml}$ ;  $P > 0.05$ ). However, insulin concentrations were significantly higher in Hispanic women with PCOS compared with PCOS Caucasian women ( $20.9 \pm 18.2\ \mu\text{IU/ml}$  versus  $10.8 \pm 3.6\ \mu\text{IU/ml}$ ;  $P < 0.05$ ). The number of Hispanic women with PCOS who also exhibited insulin resistance was higher in comparison to Caucasian women with PCOS (25% and 10%, respectively); however, the difference was not statistically significant. The difference in inhibin A concentrations in

**Table 1** Demographic data of women with polycystic ovary syndrome.

Parameter	Mean $\pm$ SD
Inhibin A (pg/ml)	4.5 $\pm$ 4.8 <sup>a</sup>
Age (years)	26.1 $\pm$ 5.2
FSH (mIU/ml)	4.1 $\pm$ 1.5
LH (mIU/ml)	10.5 $\pm$ 5.6
Glucose (mg/dl)	86.6 $\pm$ 9.1
Insulin ( $\mu$ U/ml)	10.6 $\pm$ 5.2
Testosterone (ng/dl)	63.3 $\pm$ 21.4
Free testosterone (pg/dl)	13.1 $\pm$ 4.7
17-OHP (mg/dl)	97.9 $\pm$ 59.9
DHEAS ( $\mu$ g/dl)	298 $\pm$ 234

<sup>a</sup>Inhibin A concentration was significantly lower than that of the control group (13.2  $\pm$  14.4 pg/ml;  $P = 0.003$ ).

**Figure 1** Inhibin A concentration in women with polycystic ovary syndrome compared with the control group.

PCOS women with insulin resistance compared with PCOS women who were not insulin resistant was not statistically significant.

No correlation was found between inhibin A concentration and BMI ( $r = -0.9587$ ), testosterone ( $r = 0.106323$ ), free testosterone ( $r = -0.08833$ ), DHEAS ( $r = -0.041$ ), 17-OHP ( $r = -0.1261$ ), fasting glucose ( $r = 0.014097$ ), insulin ( $r = -0.06323$ ) and insulin resistance ( $r = 0.04214$ ).

## Discussion

Inhibin A is secreted by granulosa cells and concentrations are low and relatively constant through much of the follicular phase. Inhibin A concentrations rise in the late follicular phase to reach a peak concentration in the midluteal phase. Inhibin A contributes to the suppression of FSH to nadir concentrations during the luteal phase and to the changes at the luteal follicular transition. Inhibin A may have an effect at different stages of the menstrual cycle, with serum concentrations playing a role in the fine-tuning of the pituitary secretion of gonadotrophins necessary for ovulation.

PCOS is characterized by increased inhibin B concentrations from the persistence of a large cohort of small follicles that contribute to the pool of circulating inhibins, but the pulsatile rhythm of inhibin B secretion is blunted (Lockwood

et al., 1998). In patients with PCOS who do not ovulate, it appears that inhibin A is not increased. The inhibin A concentrations are lower in the follicles of women with PCOS than in normal cohort follicles (Fujiwara et al., 2001; Magoffin and Jakimiuk, 1998). The low inhibin A concentrations found in the follicles of women with PCOS may be reflected in low serum inhibin A and may suggest an abnormality in granulosa-cell function. Further, the decrease in inhibin A concentrations in the follicular fluid of women with PCOS compared with those in the follicular fluid of size-matched follicles from normal-ovulatory women (Welt et al., 2002) suggests that inhibin deficiency may play a role in follicle arrest in women with PCOS.

This study has demonstrated that serum inhibin A concentration in patients with PCOS is lower compared with women with normal menstrual cycles of reproductive age, which is in agreement with previous findings (Pigny et al., 2000). However, Lambert-Messerlian et al. (1994) reported lower inhibin A concentrations in PCOS women which were indistinguishable from normal follicular phase. A possible explanation for the difference in findings may be the smaller sample size used in the Lambert-Messerlian study. Additionally, the current study used a commercial kit while Lambert-Messerlian et al. (1994) employed an ELISA non-commercial test to evaluate inhibin A concentrations. It should be noted that Anderson et al. (1998) indicated that treatment of PCOS patients with low doses of FSH may be sufficient to induce the development of a single dominant follicle which grows in parallel with oestradiol and inhibin A concentrations. The higher concentration of inhibin A concentrations in PCOS patients in that study may be again the result of using an ELISA non-commercial test.

This study did not uncover a correlation between inhibin A concentration and fasting glucose or insulin concentration in women with PCOS, which is consistent with results reported by Welt et al. (2002). While inhibin A concentrations in Caucasian women with PCOS were found to be not significantly higher than inhibin A concentrations found in Hispanic women with PCOS, insulin concentrations in Hispanic women with PCOS were higher, although not significantly, compared with Caucasian women. These results are consistent with previous reports indicating that elevated insulin concentrations and insulin resistance are more prevalent in Hispanic women with PCOS (Kauffman et al., 2002) and also more severe (Goodarzi et al., 2005). It should be noted that the elevated insulin concentrations and insulin resistance observed in Hispanic women with PCOS compared with Caucasian women with PCOS could possibly be due to diet, lifestyle choices or genetic variations in hormone actions such as polymorphism in gonadotrophin subunits or receptor function affecting the expression of insulin (Balen et al., 2000).

No correlation was found between inhibin A concentration and insulin, testosterone, free testosterone, 17-OHP or DHEAS concentration. The results of the present study are in agreement with Pigny et al. (2000); however, that study did not evaluate the additional androgens investigated in the current study. Previous studies (Andreani et al., 1994; Mason et al., 1994; Willis et al., 1996, 1998) suggested an abnormality of granulosa-cell function but the absence of correlation between inhibin A and androgens suggest mainly an abnormality of theca cell function.



**Table 2** Comparison between Hispanic and Caucasian women with polycystic ovary syndrome.

Parameter	Hispanic	Caucasian
No. of patients	32	10
Age (years)	26 ± 3.6	25 ± 10
BMI (kg/m <sup>2</sup> )	32.3 ± 7.8	32.6 ± 6.6
FSH (mIU/ml)	3.8 ± 1.5	4.3 ± 2.1
LH (mIU/ml)	9.9 ± 5.4	7.3 ± 4.0
Prolactin (ng/ml)	11.6 ± 6.2	11.5 ± 6.0
TSH (μIU/ml)	1.6 ± 1.0	1.9 ± 1.0
Inhibin A (pg/ml)	4.5 ± 4.9	5.1 ± 6.3
Glucose (mg/dl)	87.6 ± 21.8	76.3 ± 18.1
Insulin (μIU/ml)	20.9 ± 18.2 <sup>a</sup>	10.8 ± 3.6
Testosterone (ng/dl)	74 ± 34	69 ± 42
Free testosterone (pg/dl)	14.7 ± 6.8	12.6 ± 8.5
17-OHP (mg/dl)	90 ± 30	119 ± 103
DHEAS (μg/dl)	255 ± 186	341 ± 166
Insulin resistance <i>n</i> (%)	8/32 (25)	1/10 (10)

There were no statistically significant differences between the two groups.

BMI, body mass index; DHEAS, dehydroepiandrosterone sulphate; FSH, follicle-stimulating hormone; 17-OHP, 17-hydroxyprogesterone; TSH, thyroid-stimulating hormone.

<sup>a</sup>Values are means ± SD, unless otherwise stated.

The present study found no correlation between inhibin A concentrations and BMI which is consistent with a previous study (Pigny et al., 2000). While a relationship between low inhibin A concentrations and increased weight was identified, it was not statistically significant. Although the study did not find a correlation between inhibin A concentrations and BMI, it noted a trend that lower inhibin A concentrations were associated with increased weight. The clinical observation that PCOS women with high BMI begin to ovulate spontaneously when they lose weight may point to a relationship between weight and inhibin A concentrations.

This study suggests that serum inhibin A concentrations may have a role in the fine-tuning of pituitary secretion of gonadotrophins necessary for ovulation. In addition, lower inhibin A concentrations may prevent the antral follicles from responding to FSH thus precluding ovulation.

While this study looked at inhibin A concentrations in anovulatory women with PCOS compared with inhibin A concentrations found in normal-ovulatory women, it would be interesting to compare two anovulatory groups of women or at least an ovulatory PCOS group versus normal-ovulatory women. Further large-scale studies are needed to validate the findings presented here.

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