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Disordered follicle development

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Abstract

Alterations of ovarian follicle morphology and function have been well documented in women with PCOS. These include increased numbers of growing preantral follicles, failure of follicle growth beyond the mid-antral stage, evidence of granulosa call degeneration, and theca cell hyperplasia. Functional abnormalities include paradoxical granulosa cell hyperresponsiveness to FSH which is clinically linked to ovarian hyperstimulation during ovulation induction. In addition, there is likely a primary theca cell defect that accounts for the majority of excess androgen production in this disorder.

The precise mechanisms responsible for altered follicle function are not completely clear. However, several factors appear to influence normal advancement of follicle development as well as impair ovarian steroidogenesis. These include intra- as well as extraovarian influences that distort normal ovarian growth and disrupt steroid production by follicle cells.

Keywords

PCOS; Granulosa cell; Theca cell; Androgen; Follicle; Insulin

1. Introduction

The classical description of women with polycystic ovary syndrome (PCOS) includes evidence of excess androgen production, irregular or absent menstrual bleeding as a result of chronic anovulation, and polycystic ovaries. The lack of regular ovulatory function in these women designates PCOS as the leading cause of anovulatory infertility (Franks et al., 2008). The mechanism(s) responsible for failure of ovulation is not known, although abnormalities of the follicle and its respective cellular investments – specifically, the oocyte, granulosa cell (GC), and theca cell (TC) – have been well documented. In particular, among early and mid-antral stage follicles, the degenerative appearance of GCs on histology belies their sustained functional ability to respond to FSH and LH stimulation. Nevertheless, in women with PCOS, alterations of GC function likely contribute to the poor response to gonadotropin administration during controlled ovarian hyperstimulation. Aberrant follicle growth and development does not, however, preclude successful ovarian responsiveness to careful gonadotropin stimulation during ovulation induction. Given these observations, it is likely that extra- or intraovarian factors and not inherent defects of GCs are responsible for disruption of progressive follicle development, leading to mid-antral arrest. In contrast, a primary defect of TC steroidogenesis appears to account for androgen excess in this disorder. Whether and to what degree local androgen overproduction may impact GC

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activity is not clearly known. However, evidence suggests that androgen excess facilitates abnormal follicle formation and, perhaps, follicle viability.

2. Morphology of the polycystic ovary

The ovaries of women with PCOS are slightly enlarged and contain numerous small (2–9 mm) antral follicles, which are commonly arranged on the periphery with increased central stroma. This distinctive appearance has been codified to establish diagnostic criteria for the polycystic ovary as more than 12 follicles per ovary or an ovarian volume over 10 ml (Rotterdam, 2004a,b). Histologically, growing preantral follicles appear similar to those of normal ovaries. In addition, early antral follicle formation may also appear normal. However, normal development beyond the mid-antral stage is not observed as the follicle begins to exhibit evidence of arrested growth and degenerative change. There is progressive accumulation of follicular fluid and expansion of the antrum. As the follicle enlarges, the GC layer undergoes apoptosis and becomes increasingly atretic. Eventually, the follicle wall may become devoid of GCs, leading to the appearance of a thin-walled cyst.

3. Increased follicle number

A distinctive feature of the polycystic ovary is a pronounced increase in follicle number as the population of growing preantral and antral follicles exceed by 2–3-fold that of normal ovaries (Hughesdon, 1982; Webber et al., 2003; Maciel et al., 2004). The process responsible for excessive follicle formation in PCOS ovaries has not been established; however, several possibilities have emerged, including increased primordial follicle activation, slowed preantral follicle development, increased follicle survival and/or decreased atresia, or a combination of these processes.

3.1. Preantral follicle population

In one of the earliest descriptions of PCOS ovaries, Hughesdon found a 2-fold increase in the number of growing follicles, but the same number of primordial follicles in PCOS ovaries and normal ovaries (Hughesdon, 1982). These findings suggested that the increased number of preantral and early antral follicles in polycystic ovaries was not likely due to either an increased initial primordial pool or an accelerated rate of primordial follicle activation. Subsequent studies have shown increases in the number of total follicles; however efforts to determine the size of the primordial pool have been inconsistent. Webber et al. calculated that the number of healthy primordial follicles as a percentage of total follicles present was lower in both anovulatory and ovulatory polycystic ovaries compared to normal ovaries (Webber et al., 2003). This was interpreted to suggest that increased primordial follicle activation might contribute to the greater number of follicles in PCOS. An alternative explanation is that the growth of pre-antral follicles occurs more slowly in PCOS, resulting in an accumulation of growing follicles. This possibility is consistent with a study by Maciel et al., in which there were similar numbers of primordial follicles in PCOS and normal ovaries but a higher number of growing preantral follicles in PCOS (Fig. 1) (Maciel et al., 2004). As a result, the proportion of resting primordial follicles to the total number of growing follicles would remain decreased. Furthermore, there is no evidence that women with PCOS exhibit early menopause (Webber et al., 2003) as might be expected with accelerated primordial follicle recruitment. Taken together, these data clearly indicate that there are higher numbers of growing follicles in PCOS compared to normal ovaries with no consistent effect on primordial follicle number. However, the mechanism for this pattern remains to be determined.

3.2. Prolonged follicle survival

The observation of an increased preantral follicle population in PCOS suggests either increased entry into or decreased exit from the growing follicle pool. To investigate the latter possibility, small growing follicles were obtained from normal and PCOS women and maintained in culture for approximately 2 weeks (Webber et al., 2007). Both PCOS and normal follicles exhibited increases in diameter and growth during culture. However, examination of atresia revealed a greater percentage of degenerating follicles from normal ovaries compared to that observed in PCOS follicles. Thus, cultured PCOS follicles sustained less atresia than similar staged follicles from normal ovaries. These data suggest that slowed follicle development though the primary stage, together with a prolonged survival pattern, may lead to an accumulation of preantral follicles and further explain the greater number of growing follicles in ovaries of women with PCOS.

3.3. Role of growth and differentiation factor-9

Intriguingly, independent studies have demonstrated that the greatest increase in growing follicles in PCOS is seen in the number of follicles at the primary stage, suggesting the transition from the primary to secondary stage might be slower in PCOS (Webber et al., 2003; Maciel et al., 2004). A possible explanation for slowed growth at the primary follicle stage was provided by Teixeira Filho et al. (2002). In PCOS and normal ovaries they examined the expression of growth differentiation factor-9 (GDF-9), an oocyte-specific member of the TGF-β family known to play a critical role in folliculogenesis. GDF-9 expression was decreased in PCOS ovaries compared to normals in oocytes at all developmental stages from transitional to the Graafian follicles. Studies in mice have suggested that adequate amounts of GDF-9 are required for growth beyond the primary follicle as female GDF-9 knock-out mice are infertile with a complete block in folliculogenesis at the primary stage (Dong et al., 1996). Filho et al. showed that only 8–12% of the oocytes of primary follicles in PCOS contained signal for GDF-9 compared to 96% of normal controls, and the signal, when present in more advanced follicles, was weaker than corresponding normal follicles (Teixeira Filho et al., 2002). Thus, defects in growth in PCOS may begin at the earliest stages of folliculogenesis, well before arrest of the antral follicle.

3.4. Anti-mullerian hormone

Abnormal expression of anti-mullerian hormone (AMH) in PCOS may also play a role in increased follicle density. This TGF-beta peptide is produced exclusively by GCs of growing pre-antral and small antral follicles and serves to negatively regulate advancement of follicle maturation (Weenen et al., 2004). In primordial and primary follicles from PCOS ovaries, AMH expression was decreased compared with that of normal ovaries (Stubbs et al., 2005). The relative diminution of AMH expression in early stage follicles may provide a possible mechanism for increased numbers of preantral follicles in PCOS. Interestingly, in women with PCOS serum AMH levels are elevated 2–3-fold compared to values observed in normal women. While this has been attributed to increase follicle number in PCOS, recent evidence indicates that *in vitro* AMH production per GC is increased in this disorder (Pellatt et al., 2007). In other words, each small antral follicle from PCOS ovaries makes more AMH than a corresponding normal follicle. Collectively, these findings suggest that in women with PCOS AMH expression is reduced in early growing follicles, which might allow for advanced development. However, with advanced follicle growth to the antral stage, increased AMH production occurs as a result of increased follicle number as well as greater output on a per cell basis.

3.5. Androgen

Among local factors that might accelerate follicle growth, androgen excess is suggested from polycystic ovary morphology associated with hyperandrogenic women with congenital adrenal hyperplasia or androgen-producing ovarian tumors (Dunaif et al., 1984; Erickson et al., 1989; Barnes et al., 1994). Moreover, female-to-male transsexuals receiving long-term androgen treatment commonly exhibit polycystic ovary morphology (Futterweit and Deligdisch, 1986; Spinder et al., 1989; Chadha et al., 1994). Similar findings have been observed in nonhuman primates administered high doses of testosterone, where increased ovarian size was accompanied by greater numbers of preantral and antral follicles (Vendola et al., 1998). In addition, it was shown that androgen induced increased FSH receptor expression in GCs, which suggests a mechanism for this androgen effect.

4. Arrest of follicle development

It has been recognized that, in PCOS, follicle development fails to proceed beyond the mid-antral stage. Based on observational data, arrest of follicle growth typically occurs when follicular diameter reaches 5–8 mm. However, the mechanism is responsible for arrest or precisely when cessation of follicle development is initiated is not known. Possible mechanisms that have been suggested include premature terminal GC differentiation and insufficient FSH secretion.

4.1. Premature luteinization

In a series of elegant studies, steroid production from GCs of individual PCOS and normal follicles of varying size were assessed following LH stimulation (Willis et al., 1998). LH-stimulated progesterone (P_4) production from small PCOS follicles (4 mm) was greater or, at least, equivalent to the amount of P_4 generated by larger follicles from normal ovaries (9.5–10 mm). These findings suggested that GCs in follicles from PCOS ovaries had acquired LH receptors at an earlier stage of development and, subsequently, undergone luteinization prematurely. That LH secretion in women with PCOS is increased only underscores the likelihood of this mechanism as a prelude to terminal differentiation and arrested follicle development. This concept was reinforced by studies that revealed overexpression of LH receptor mRNA in PCOS GCs compared to GCs from normal follicles (Jakimiuk et al., 2001).

Precisely how GCs of small antral follicles might acquire increased LH receptors is unknown. It is well established that FSH induces LH receptor content in cultured GCs (Richards, 1994; Lindeberg et al., 2007). However, in women with PCOS, FSH secretion is reduced. Insulin has been shown to stimulate FSH-induced LH receptor in rat and porcine GCs although inhibition of LH receptors by insulin has also been reported (May et al., 1980; Sanders and Midgley, 1982; Davoren et al., 1986). The relevance of these findings in PCOS is unclear as androgen has been shown to inhibit FSH-induced LH receptor expression in cultured rat GCs (Jia et al., 1985). Nevertheless, in GCs from PCOS follicles, insulin and LH were synergistic in stimulating steroid production (Willis et al., 1996; Franks et al., 2008). These results raise the possibility that increased insulin secretion in women with PCOS promotes LH action on GCs, and by extension, contributes to follicle atresia and eventual arrest.

4.2. Inadequate FSH secretion

Failure of follicle maturation in PCOS has also been attributed to inadequate FSH stimulation as serum levels of FSH are decreased in women with PCOS. Yet, GCs of PCOS express increased FSH binding and are readily responsive to FSH *in vitro* and *in vivo* (Almahbobi et al., 1996; Willis et al., 1996; Coffler et al., 2003a). Additionally, it is well

established that anovulation in PCOS women may be overcome with exogenous FSH administration. The relative suppression of FSH secretion, as well as increased LH secretion, may be a consequence of increased activity of the hypothalamic GnRH pulse generator observed in women with PCOS. LH and FSH sub-unit genes are differentially regulated as increased GnRH pulse activity inhibits FSH-β gene expression and increases LH-β gene expression, thereby accounting for inappropriate gonadotropin secretion PCOS (Kaiser et al., 1995). Further reduction of circulating FSH may also result from the chronic estrogen secretion characteristic of this condition. In PCOS women treated with estrogen over a two week interval, serum FSH levels declined progressively and the disparate release of the gonadotropins FSH and LH was enhanced (Chang et al., 1982).

4.3. Role of anti-mullerian hormone

Because AMH inhibits aromatase, it has been proposed that excessive local production of this protein, combined with reduced FSH secretion, could set the stage for follicle arrest and decreased estradiol (E_2) production by the ovary (Grossman et al., 2008). This notion is consistent with *in vitro* studies showing that GCs incubated for 48 h in the presence of FSH exhibited reduced AMH production (Pellatt et al., 2007). In addition, during controlled ovarian hyperstimulation, serum AMH levels have been noted to decline with length of treatment (Fanchin et al., 2003; La Marca et al., 2004). However, these observations may also be explained by the decrease in AMH production seen in the final stages of follicle development in normal ovaries. The possible role of AMH in follicle arrest in PCOS is uncertain as endogenous factors that regulate this protein are currently unknown.

5. Granulosa cell steroidogenesis

Despite the observed degenerative changes, the functional integrity of GCs of PCOS follicles is retained and, to a significant extent, is even greater than that of normal GCs. Early *in vitro* studies demonstrated that E_2 production by PCOS GCs following FSH stimulation was 6- to 10-fold greater compared to responses observed in normal cells (Erickson et al., 1990; Mason et al., 1994). This enhanced responsiveness was found predominantly in cells from anovulatory PCOS women and not hyperandrogenic women with ovulatory cycles.

Subsequent clinical studies have been consistent with the results of these *in vitro* studies. When women with PCOS were acutely stimulated with increasing doses of intravenous FSH, the highest dose of FSH produced significantly greater E_2 production than that observed in normal women (Fig. 2) (Coffler et al., 2003a). Interestingly, the time-course response revealed that, in PCOS women, increases of E_2 were not sustained as peak levels were followed by an estimated 40% decrement while E_2 levels in normal women persisted for 24 h after reaching maximal values. Thus, while hyperresponsive to FSH, PCOS GCs were unable to sustain E_2 production, perhaps reflecting the underlying degenerative process of GC attrition in these cells.

The mechanism by which GC sensitivity to FSH is increased in PCOS has not been clearly defined. Previous studies in rodents and non-human primates have shown that E_2 production in GCs may be enhanced by several factors including insulin, estrogen, and androgen. These factors may contribute to increased GC sensitivity to FSH as women with PCOS exhibit chronic unopposed estrogen secretion, excessive androgen production, and insulin resistance with compensatory hyperinsulinemia.

5.1. Role of insulin

To address the role of insulin on GC function, a series of meticulous studies were performed on GCs obtained from ovaries of PCOS and normal women. Incubation with insulin alone

was associated with increased E_2 and P_4 production from GCs of normal ovaries as well as those from ovulatory and anovulatory PCOS ovaries (Willis et al., 1996). When combined with FSH, there was an additive effect of insulin on normal and ovulatory PCOS GCs whereas a synergistic response to these combined hormones was exhibited by GCs from anovulatory women with PCOS. These findings are consistent with the observation that insulin resistance is more pronounced in anovulatory compared to ovulatory PCOS individuals and imply a role for insulin in GC steroidogenesis in this group of women.

To determine the clinical effect of insulin on GC responses to FSH, anovulatory PCOS and normal women were administered a 10-h hyperinsulinemic-euglycemic clamp combined with FSH stimulation (Coffler et al., 2003b). After induced hyperinsulinemia, E_2 responses to FSH were unaltered compared to responses observed without insulin infusion (Fig. 3). Subsequently, the PCOS women were treated with an insulin lowering drug, pioglitazone, for 12 weeks, after which insulin infusion and FSH stimulation were repeated. Serum E_2 responses to FSH were significantly greater than that observed before treatment (Fig 3). In addition, it was also noted that with piogltiazone, the duration of maximal E_2 response was more sustained and similar to that observed in untreated normal women. Notably, initial E_2 responsiveness to FSH was more rapid and of greater magnitude compared to that occurring without insulin infusion. These results suggest that hyperinsulinemia may not contribute to enhanced E_2 production in PCOS women, but that initial GC responsiveness to FSH may be improved by reducing insulin resistance, as might occur during ovulation induction.

As previously mentioned, cultured PCOS GCs appeared to be prematurely luteinized based on P_4 responsiveness to LH and FSH stimulation (Willis et al., 1998). In women with PCOS we have examined P_4 responses to FSH before and after hyperinsulinemia. Under basal conditions, women with PCOS exhibited significantly increased FSH-stimulated P_4 production whereas incremental changes in normal women were not apparent. Insulin infusion failed to impact P_4 responses to FSH. In addition, treatment with pioglitazone which improved insulin sensitivity was not associated with greater P_4 responses either prior to or during a hyperinsulinemic clamp compared to pretreatment responses. The observation of greater P_4 responses to FSH in PCOS women compared to that of normal women is consistent with prematurely luteinized GCs in this disorder. However, absence of an effect of hyperinsulinemia suggests that insulin has a limited, if any, role in FSH-mediated P_4 release in PCOS women.

5.2. Role of androgen

Co-incubation of androgen with GCs from rodents and non-human primates has been shown to enhance estrogen responses to FSH stimulation (Erickson and Hsueh, 1978; Daniel and Armstrong, 1980; Hillier and De Zwart, 1981; Harlow et al., 1986). This ability of androgen to enhance GC function is not limited to aromatizable compounds as the non-aromatizable androgen, dihydrotestosterone, produced the same effects. Subsequent studies have revealed that, in GCs of non-human primates with implanted silastic capsules containing testosterone, FSH receptor expression was increased and co-localized with that of the androgen receptor (Vendola et al., 1998; Weil et al., 1999). Thus, excess androgen production may contribute to greater ovarian responsiveness to FSH in hyperandrogenic women with PCOS.

In an indirect attempt to assess the influence of hyperandrogenemia on GC steroid production, E_2 responses to FSH stimulation were assessed in women with PCOS prior to and following flutamide treatment for 6 weeks (Mehta et al., 2006). Baseline and maximally stimulated E_2 responses were not affected by flutamide treatment. Among androgen responses, the only significant change was decreased serum DHEA-S levels, which denoted bioeffectiveness of flutamide administration. These findings indicate that, contrary to

findings *in vitro*, GC hyperresponsiveness to FSH observed in women with PCOS may not be attribus to increased circulating androgens.

5.3. Role of estrogen

It has been previously shown that estrogen can enhance ovarian responses to FSH *in vitro* (Richards et al., 1979; Adashi and Hsueh, 1982; Jonassen et al., 1982; Daniel and Armstrong, 1983). In cultured GCs, synergy between E_2 and FSH has been demonstrated with regards to increased FSH receptor binding, induction of LH receptors, increased aromatase activity, and progestin synthesis (Richards et al., 1976; Adashi and Hsueh, 1982). The mechanism for this synergy has not been completely elucidated although an effect on adenylate cyclase or estrogen-induced GC proliferation was suggested since FSH receptor expression did not appear to be altered (Richards, 1980; Daniel and Armstrong, 1983). Alternatively, other investigators have demonstrated increased FSH-binding capacity per GC following stimulation by FSH using estrogen-treated immature rats (Knecht et al., 1984). Both processes may serve to amplify FSH receptor number in GCs of developing follicles. In humans, increased FSH binding to GCs derived from antral follicles of anovulatory women with PCOS compared to those of sized-matched follicles from normal ovaries has been reported (Almahbobi et al., 1996). The precise mechanism for greater FSH binding is not obvious.

In an attempt to show whether estrogen may influence GC responsiveness to FSH in women with PCOS, we examined E_2 responsiveness to FSH during ovarian recovery from GnRH agonist administration before and after estrogen supplementation (unpublished data). Maximal suppression at 4 weeks was followed by a gradual rise of basal and stimulated E_2 levels returning to pretreatment values by 8 weeks (Fig. 4). Subsequently, the protocol was repeated with application of transdermal estrogen 2 weeks after GnRH agonist injection to provide serum E_2 levels comparable to basal values observed in PCOS women. In E_2-treated women, FSH-stimulated E_2 production during recovery was unchanged from maximally suppressed values at 4 weeks post-GnRH agonist. Thus, early restoration of estrogen exposure following ovarian suppression did not accelerate GC responses to FSH during recovery. However, the concentrations achieved with transdermal E_2 likely inhibited recovery of gonadotropin secretion and reflected extreme sensitivity of GnRH-FSH interaction to negative E_2 feedback, much like that which occurs during early puberty.

6. Theca cell androgen production

Evidence of normal TC growth is initially seen during secondary follicle development as thin, elongated stromal cells begin to emerge next to the basement membrane and surround the follicle. The mechanism by which follicles acquire TCs is not known. With continued growth of the follicle, the TCs differentiate into the theca interna, which has steroidogenic capacity and the ability to generate androgens. Unlike the appearance of normal TCs, antral follicles of polycystic ovaries are surrounded by a theca layer of considerably greater cellularity and thickness, usually exceeding the width of the corresponding GC layer (Erickson and Yen, 1984). Little is known about the genesis of theca hyperplasia. Evidence indicates that abnormally increased pituitary LH secretion certainly plays a role in excess ovarian androgen production (Rebar et al., 1976; de Ziegler et al., 1989). However, there is now substantial evidence to also suggest a primary defect in TC steroidogenesis in PCOS, leading to excessive responses to gonadotropin stimulation. Intriguingly, gonadotropins appear to act both directly and indirectly to influence androgen production by TCs.

6.1. Role of insulin

In PCOS, the most notable extraovarian factor implicated in androgen overproduction is insulin. The association between hyperinsulinemia and hyperandrogenemia in women with PCOS might be explained by the finding that the activity of a single serine kinase may result in both hyperandrogenemia and insulin resistance as it appears to phosphorylate CYP17, activating androgen production, as well as the insulin receptor-β, blocking insulin signaling (Dunaif et al., 1995; Bremer and Miller, 2008). However, *in vitro* studies suggest a more direct relationship exists. Studies involving culture of normal human theca tissue have demonstrated that insulin is capable of enhancing androgen production in response to LH as well as independently stimulating androgen production (Barbieri et al., 1984, 1986; Bergh et al., 1993). In TCs from women with PCOS, insulin was shown to markedly increase androgen production. The relationship *in vivo*, however, is less clear. The reduction of hyperinsulinemia in women with PCOS treated with insulin-lowering drugs is associated with significant decreases of serum androgen levels without corresponding changes in LH levels. This observation indirectly suggests a role for insulin in LH-stimulated androgen synthesis in women with this disorder. However, induction of marked hyperinsulinemia in women with PCOS has not been associated with increases in serum androgen levels (Nestler et al., 1987; Dunaif and Graf, 1989). More studies are needed to resolve these discrepant results.

6.2. Primary theca cell defect

There is good evidence to suggest that excess ovarian androgen results from a primary defect of TC steroidogenesis in women with PCOS. Initial studies established that cultured TCs from PCOS ovaries produced significantly more androgen both at baseline and following LH stimulation per cell than that generated by TCs from normal ovaries (Barbieri et al., 1984; Gilling-Smith et al., 1994). To determine the nature of excess ovarian androgen production *in vivo*, GnRH agonist was administered to women with PCOS, normal women and normal men followed by frequent blood sampling over 24 h (Barnes et al., 1989). PCOS women exhibited significant increases in serum 17-hydroxyprogesterone (17-OHP) and androstenedione (A_4) concentrations compared to those of normal women. Moreover, these responses were similar to those observed in normal men. Interestingly, differences in testosterone (T) responses between women with PCOS and normal women were not found. Examination of the patterns of individual steroid hormone responses pointed to alterations in both 17-hydroxylase and C-17, 20-lyase activity. As CYP17 regulates the activity of enzymes, it was proposed that androgen excess may arise *de novo* from the ovary as a result of dysregulation of CYP17 activity in PCOS. Consistent with this hypothesis, a subsequent study of PCOS women showed that 17-OHP production in response to human chorionic gonadotropin (hCG), a surrogate for LH stimulation, was elevated compared to that of normal women and remained amplified following suppression of endogenous gonadotropins by GnRH agonist (Gilling-Smith et al., 1997). In contrast, the post-GnRH agonist response in normal women was significantly lowered. Taken together, these clinical results support the concept of an inherent dysregulation of CYP17 in TCs leading to increased androgen production in response to gonadotropin stimulation in women with PCOS.

While PCOS TCs are more responsive on a per cell basis *in vitro*, it was still unclear whether TCs were more sensitive to LH secretion in women with PCOS compared to normal women. To further investigate this possibility, we conducted a dose–response study in PCOS and normal women to determine androgen production following intravenous administration of hCG at doses of 1, 10, 25, 100, and 250 μg (Rosencrantz et al., 2011). Our results revealed that 17-OHP responsiveness to hCG increased in a dose dependent manner in PCOS women and was greater than that observed in normal women (Fig. 5). A shift in the response curve was not observed, which indicated greater TC responsiveness and not

increased sensitivity to hCG. Additionally, it was noted that peak A_4 and T responses in PCOS women occurred at a low dose of hCG whereas maximal 17-OHP responses were observed at higher doses of hCG. Interestingly, hCG-stimulated responses of A_4 and T in normal women were not observed. These results suggested exquisite TC responsiveness to hCG in PCOS women compared to minimal stimulated androgen production in normal women. Previous clinical studies have suggested a lack of 17, 20-lyase activity based on GnRH-stimulated rises of A_4 that were relatively less than that of 17-OHP (Rosenfield et al., 1994). Using propagated PCOS TCs, it was demonstrated that radiolabeled pregnenolone was more rapidly metabolized to 17-OH pregnenolone and DHEA and, eventually, to A_4 compared to normal cells (Nelson et al., 1999). This increased steroid output was accompanied by greater enzymatic activities of CYP11A, 3β-HSD, and 17β-HSD. The inefficiency of del-ta-4 17, 20-lyase activity may explain, at least in part, the discordant dose–responses of 17-OHP compared to those exhibited by A_4 and T in PCOS women. The accumulation of A_4 and T with minimal 17-OHP production at a low dose of hCG is compatible with hyperactivity of the delta-5 steroid pathway combined with increased 3β-HSD enzyme activity, a stable property of the PCOS theca cell (Nelson et al., 1999). At higher doses of hCG, incremental changes in 17-OHP responsiveness likely arose from combined over-expression of both 17-hydroxylase and 3β-HSD enzymes.

6.3. Role of LH secretion

To further determine the contribution of endogenous LH to androgen production, we examined TC response to IV hCG stimulation in PCOS and normal women prior to and 2 days following administration of a GnRH antagonist (unpublished data). Antagonist treatment significantly lowered LH and moderately decreased FSH. As expected, basal estrogen and androgen levels were also significantly reduced (Fig. 6). Prior to antagonist injection, hCG stimulated clear increases of serum 17-OHP, A_4, and T in PCOS women while changes in normal women were not significant. After LH suppression by antagonist administration, both groups demonstrated reduced basal levels of androgens. Accordingly, peak androgen responses to hCG were lower in each group. However, the incremental fold-change for both PCOS and normal women was unaltered. Our results are not surprising as amplified 17-OHP responses to GnRH agonist stimulation in PCOS women were reported to be unaltered despite ovarian suppression for one month (Gilling-Smith et al., 1997). In addition, after 3 months of ovarian suppression by long acting GnRH agonist, women with PCOS exhibited 17-OHP responses to hCG that remained greater than those of normal women albeit at substantially lower circulating values (Hirsh-feld-Cytron et al., 2009). Collectively, these results suggest that endogenous gonadotropin secretion contributes to basal androgen secretion and, even in the presence of lowered gonadotropin levels, maintains ovarian androgen synthesis and responsiveness to gonadotropin stimulation. This is consistent with primary TC dysfunction leading to excessive ovarian androgen production in women with PCOS.

7. Intrafollicular paracrine interaction

We have previously examined the pattern of hormone recovery following ovarian suppression by GnRH agonist in women with PCOS. As expected, we noted maximal suppression at 4 weeks after agonist administration followed by recovery of steroidogenesis, with serum E_2 levels returning to pretreatment values 8 weeks (Fig. 4). Basal serum A_4 and T levels, as well as basal FSH levels, were restored to pretreatment values by 6 weeks. In contrast, serum LH remained significantly suppressed at 6 weeks and only resumed pretreatment levels at 8 weeks. These results demonstrated that recovery of ovarian steroidogenesis was marked by an accelerated return of androgen production that was more rapid than that of estrogen and coincided with the rapid return of FSH.

The coupling of androgen and FSH recovery patterns, particularly in the absence of restored LH secretion, suggested that FSH may induce TC androgen production. To address this possibility, androgen responses to FSH were tested in women with PCOS and normal women. In the PCOS group, significant, incremental increases in 17-OHP, A_4, and DHEA following FSH stimulation were observed whereas androgen responses to FSH were unchanged in normal women (Fig. 7) (Wachs et al., 2008). By comparison, FSH provoked a significant reduction in T production in both groups, which might reflect conversion to E_2 by FSH-activated aromatase. Surprisingly, these findings provide evidence that, in PCOS women, TC androgen production is enhanced by FSH administration and suggest a paracrine mechanism by which GC factors produced in response to FSH stimulate TC androgen production.

7.1. Inhibin

In vitro and *in vivo* studies in animals have shown that, among factors produced by GCs, inhibin may enhance LH-stimulated TC androgen production (Hillier and De Zwart, 1981). However, reports of serum inhibin B levels in women with PCOS have been inconsistent in that some have shown increased concentrations and others no change (Magoffin and Jakimiuk, 1998; Laven et al., 2001; Welt et al., 2005). These conflicting findings were clarified by examining inhibin B and E_2 responses to FSH stimulation in PCOS and normal women. In women with PCOS, inhibin B levels rose 5-fold after FSH (Fig. 8), which was significantly greater than the 3-fold increase observed in normal women (Wachs et al., 2006).

A direct effect of inhibin B on TC androgen production is unlikely as a receptor for this protein has not been identified. Rather, inhibin B has been shown to bind a membrane bound protein, beta-glycan, to form a complex that has high affinity for activin (Act) type II receptors (Lewis et al., 2000). Sequestration of Act type II receptors prevents formation of the Act type II/type I receptor complex, activin signaling, and inhibition of CYP17 in the TC. This provides a possible mechanism for FSH-stimulated androgen production in PCOS women. However, documentation of this interaction in human ovarian tissue and whether inhibin/activin signaling is altered in TCs of PCOS women has not been studied.

7.2. Bone morphogenetic proteins

Other factors that utilize Act type II receptors may also be inactivated through the competitive action of inhibin B. In particular, some bone morphogenetic proteins (BMPs) bind to not only BMP type II receptors, but also Act type II receptors to initiate their divergent actions (Shimasaki et al., 2004). It has been reported BMPs may inhibit CYP17 mRNA expression and androgen production in ovarian TC culture (Dooley et al., 2000; Glister et al., 2005). Whether the inhibitory effect of BMPs on androgen production involves BMP or Act type II receptors is unknown, however, the possibility that inhibin B may act to antagonize BMP signaling through binding to beta-glycan is an intriguing hypothesis under investigation (Wiater and Vale, 2003).

7.3. Insulin-like growth factors

In addition to inhibin B, insulin-like growth factors (IGF) I and II also may act to influence TC androgen production (Cara and Rosenfield, 1988; Bergh et al., 1993; Nahum et al., 1995). *In vitro*, IGF-I has been shown to synergize with LH to enhance androgen production from cultured rodent TCs (Hillier et al., 1991). However, because IGF-I mRNA and protein do not appear to be expressed in human GCs, it is more likely that IGF-II is the active paracrine factor. In human TC tissue, IGF-II was shown to stimulate basal androgen release as well as enhance LH-induced androgen production much like that of IGF-I (Nahum et al., 1995). In the same study, the stimulatory effects of IGF-I and IGF-II on TC androgen

production were greatly enhanced by inhibin B, which supports a predominant role for this protein among potential GC mediators of TC androgen production in PCOS.

8. Summary

Disordered follicle development is a cardinal feature of women with PCOS. The distinctive polycystic ovary morphology is accompanied by alterations in steroid hormone production that have been characterized *in vitro* as well as *in vivo*. However, the precise mechanisms responsible for increased preantral follicle number, arrested follicle growth, and abnormal steroidogenesis remain incompletely understood. Clearly, there are inherent defects within the ovary of women with PCOS although evidence for disrupted GC/TC paracrine interaction and roles for extraovarian factors are compelling.

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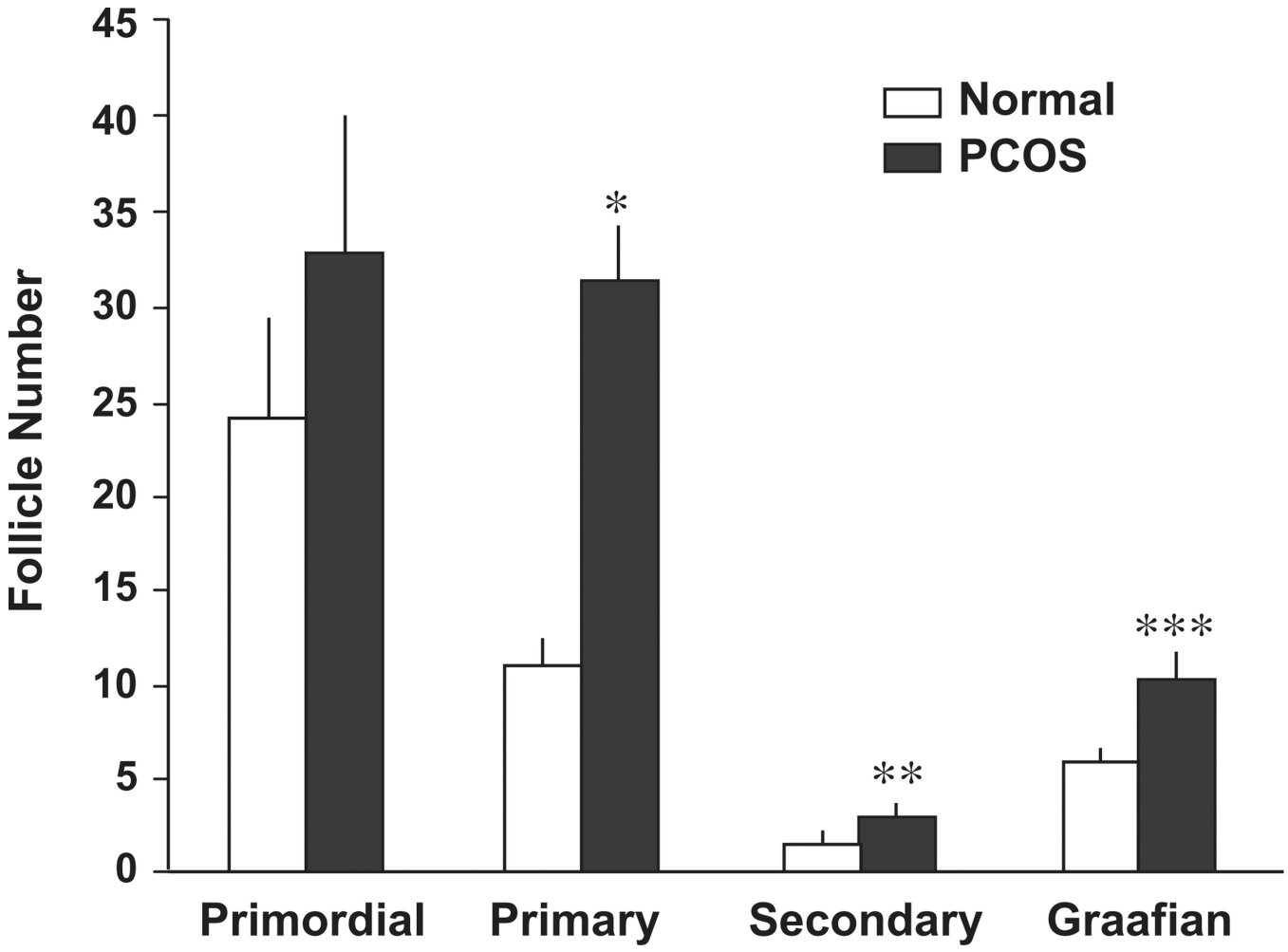

Fig. 1.

The size (mean ± SE) of the follicle population (primordial, primary, secondary, and Graafian) in sections of ovaries from normal and PCOS patients. *$P = 0.001$; **$P = 0.02$; ***$P < 0.001$.

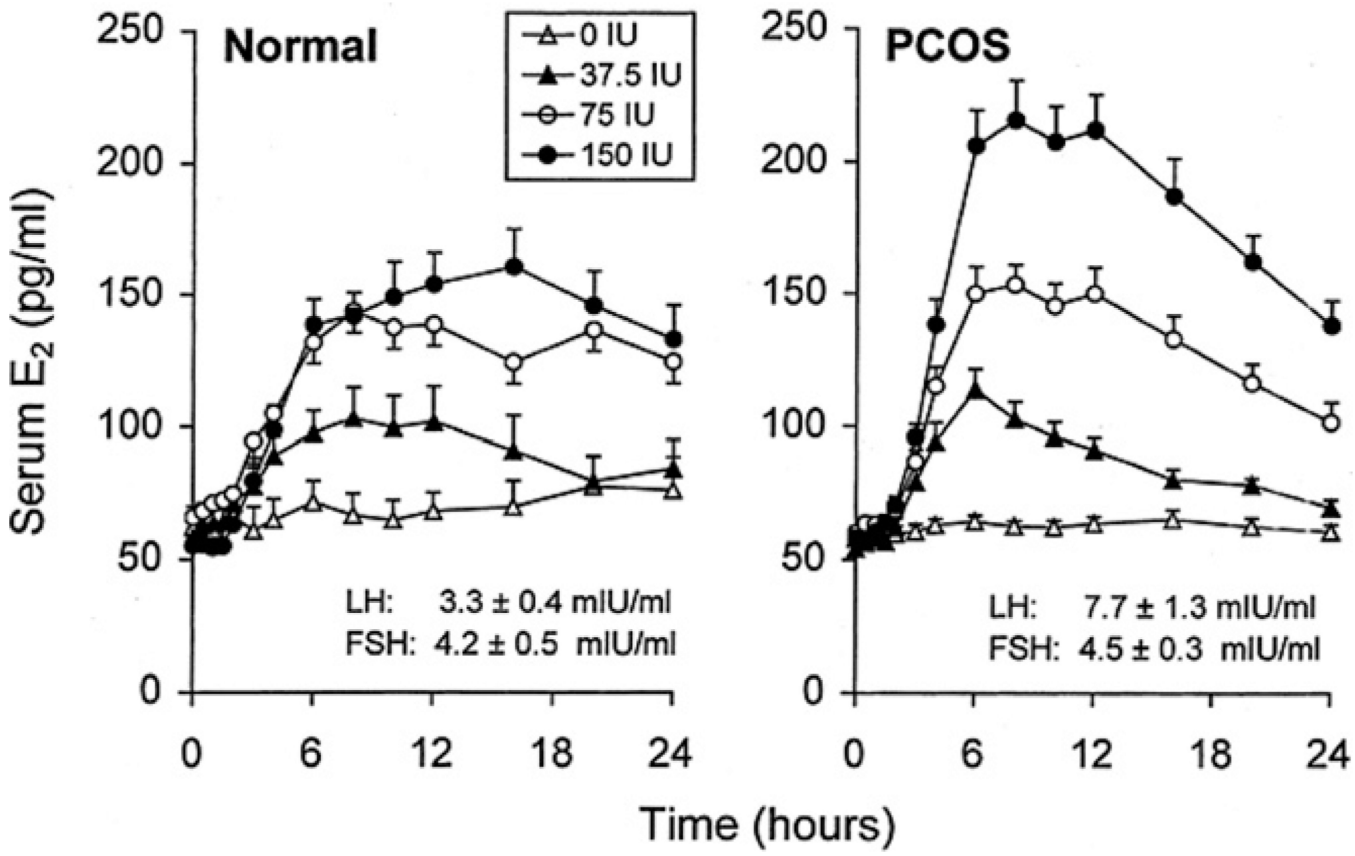

Fig. 2.

Mean (±SE) serum E_2 levels after intravenous administration of FSH in PCOS and normal controls. FSH administered after t = 0 h. The 0 IU dose of FSH is saline control. Mean (± SE) baseline levels of serum LH and FSH are also shown. (Conversion of E_2 to SI units by factor of 3.67).

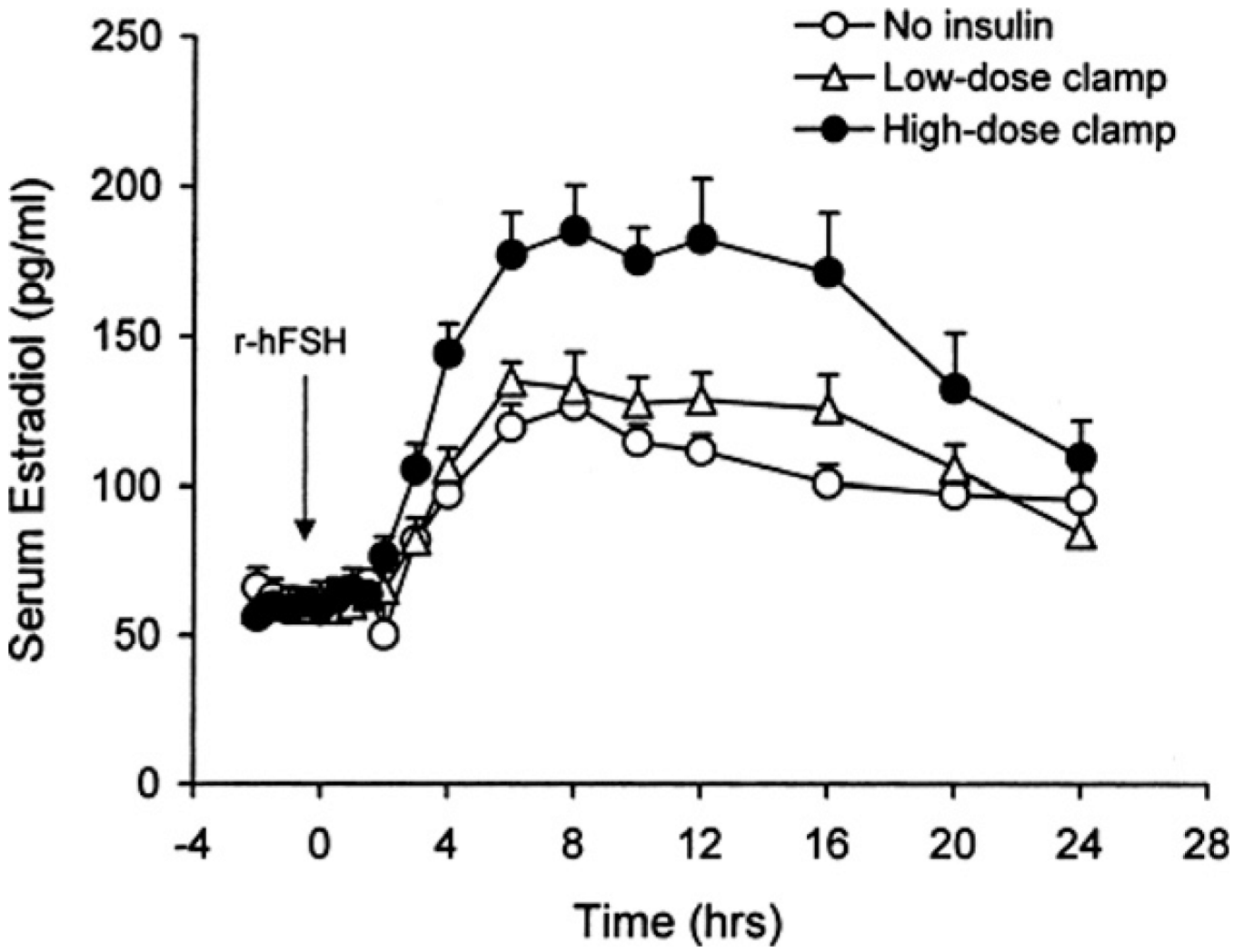

Fig. 3.

Time course of mean (±SE) 24 h serum E_2 responses after injection of intravenous FSH, 75 IU, to PCOS women treated with pioglitazone without insulin infusion and 2 h after initiation of low-dose and high-dose hyperinsulinemic-euglycemic clamps administered for 10 h. The integrated E_2 response as determined by area under the curve was significantly greater in subjects receiving high-dose insulin infusion compared with those observed for women without insulin or with low-dose insulin infusion. (Conversion of E_2 to SI units by factor of 3.67).

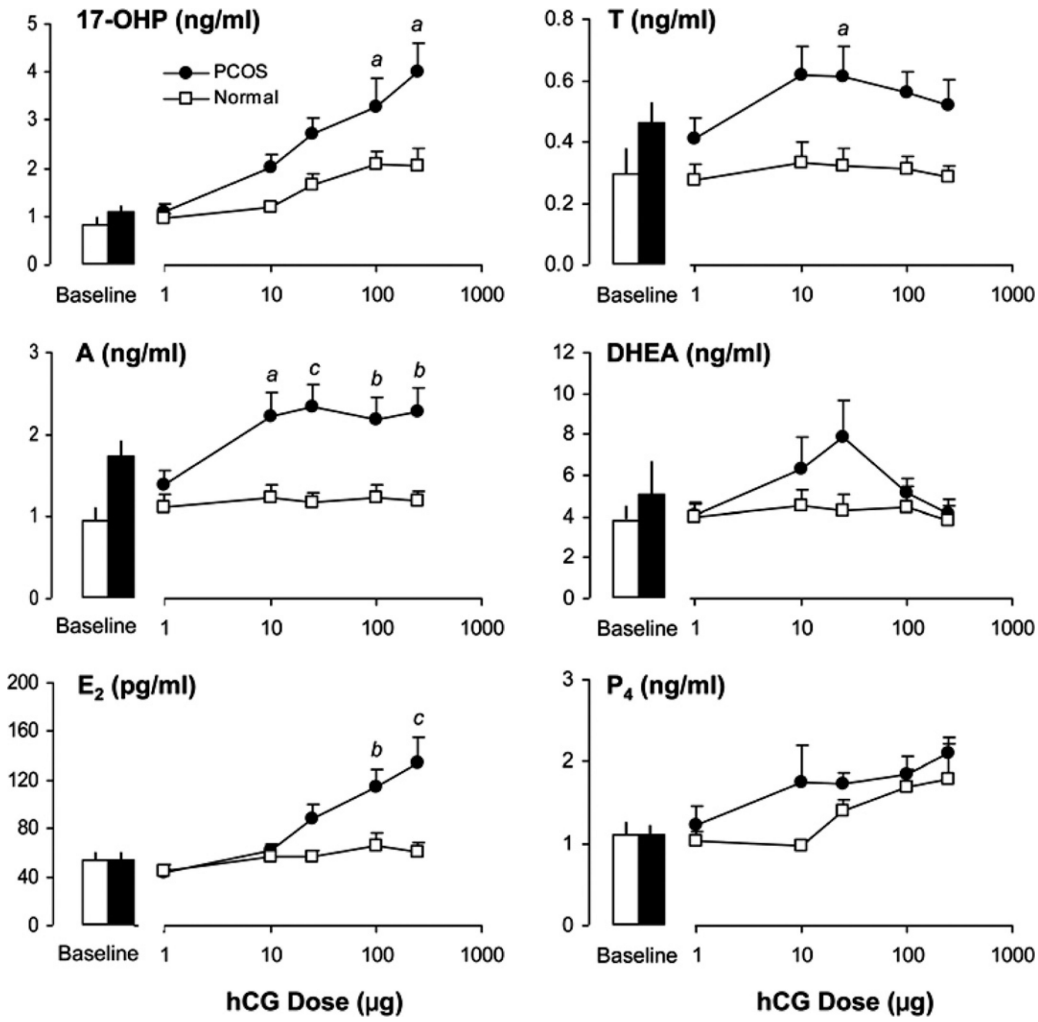

Fig. 4.

Mean (±SE) baseline and 24-h serum 17-OHP, A_4, T, DHEA, E_2, and P_4 responses to intravenous administration of 1, 10, 25, 100, and 250 μg of hCG in PCOS and normal women. Using linear mixed-effect models analyses, significant increases of 17-OHP, E_2, and P_4 were observed in both groups. By comparison, incremental A responses were significant only in PCOS women. Between groups, 17-OHP and A responses were significantly greater in PCOS women. Serum T responses were also higher compared to normal women, but the difference did not achieve statistical significance. Significant differences between groups in response to a fixed dose of hCG are indicated by a ($P < 0.05$), *b* ($P < 0.01$), and *c* ($P < 0.001$). (Conversion of 17-OHP, A_4, T, DHEA, E_2, and P_4 to SI units by factors of 3.03, 3.49, 3.47, 3.47, 3.67, and 3.18, respectively).

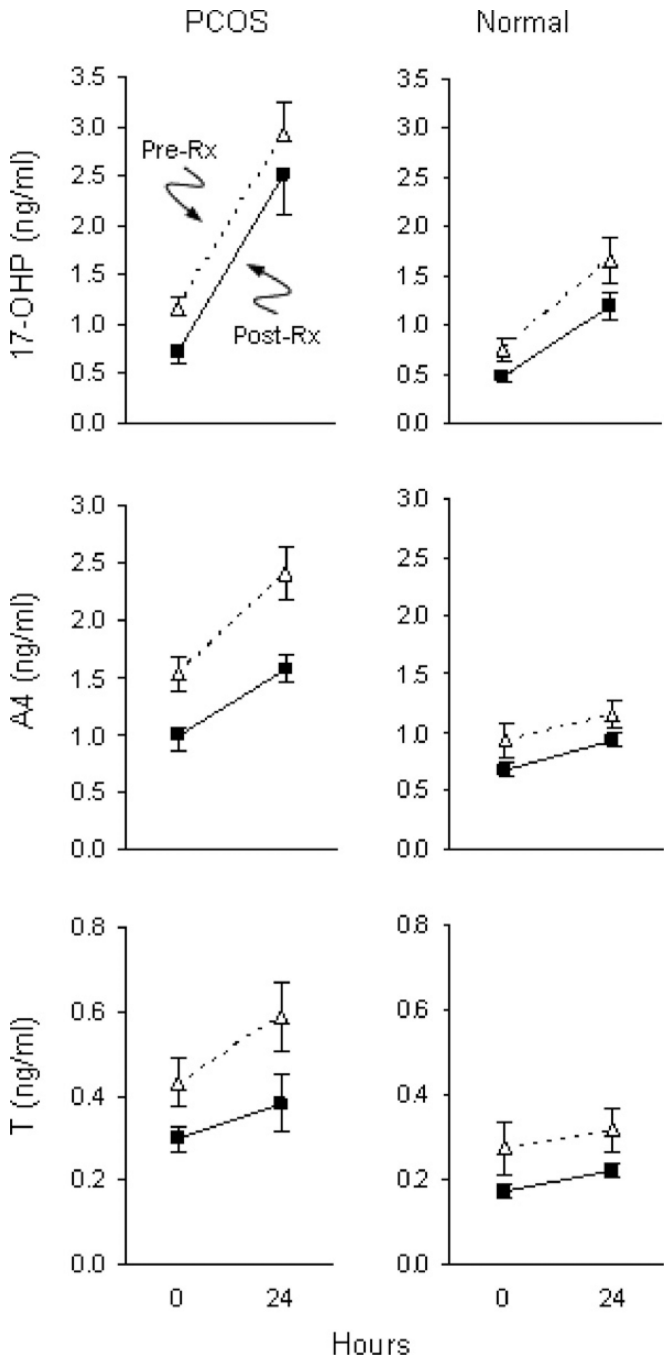

Fig. 5.

Mean (±SE) serum 17-OHP, A4, and T levels following administration of hCG, 25 μg intravenously, prior to and after treatment with GnRH antagonist in women with PCOS and normal controls. The rise from baseline values is significant ($P < 0.05$) for all pre- and post-treatment responses in both groups except for the T response in normal women before GnRH antagonist. (Conversion of 17-OHP, A, T, to SI units by factors of 3.03, 3.49, and 3.47, respectively).

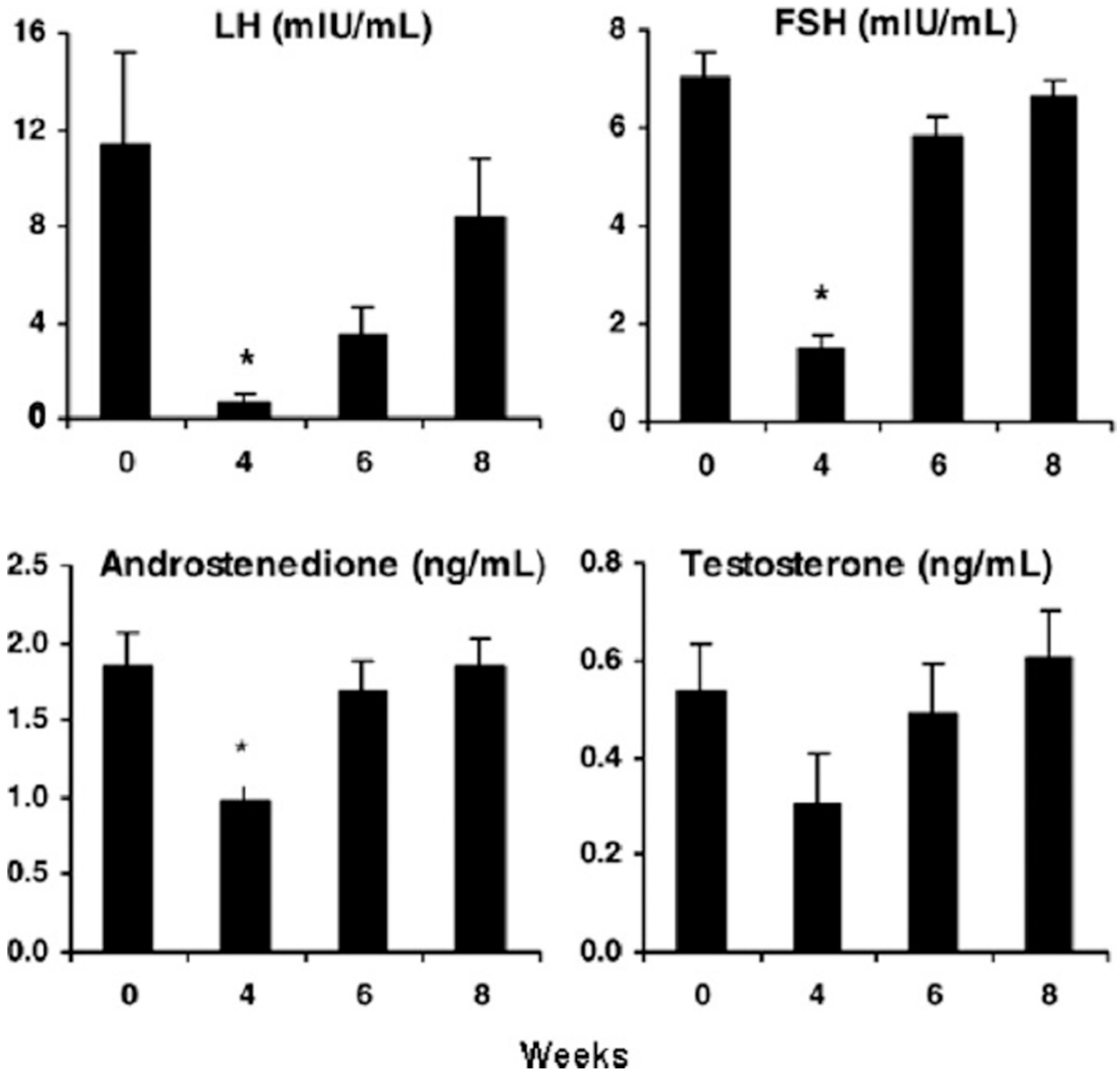

Fig. 6.

Mean (±SE) basal serum levels of LH, FSH, A and T before and 4, 6 and 8 weeks after treatment with a single injection of a long-acting GnRH agonist (depot Lupron, 3.75 mg, i.m.) in six PCOS women. *$P < 0.05$ versus 0 weeks. (Conversion of A_4 and T to SI units by factors of 3.49 and 3.47, respectively).

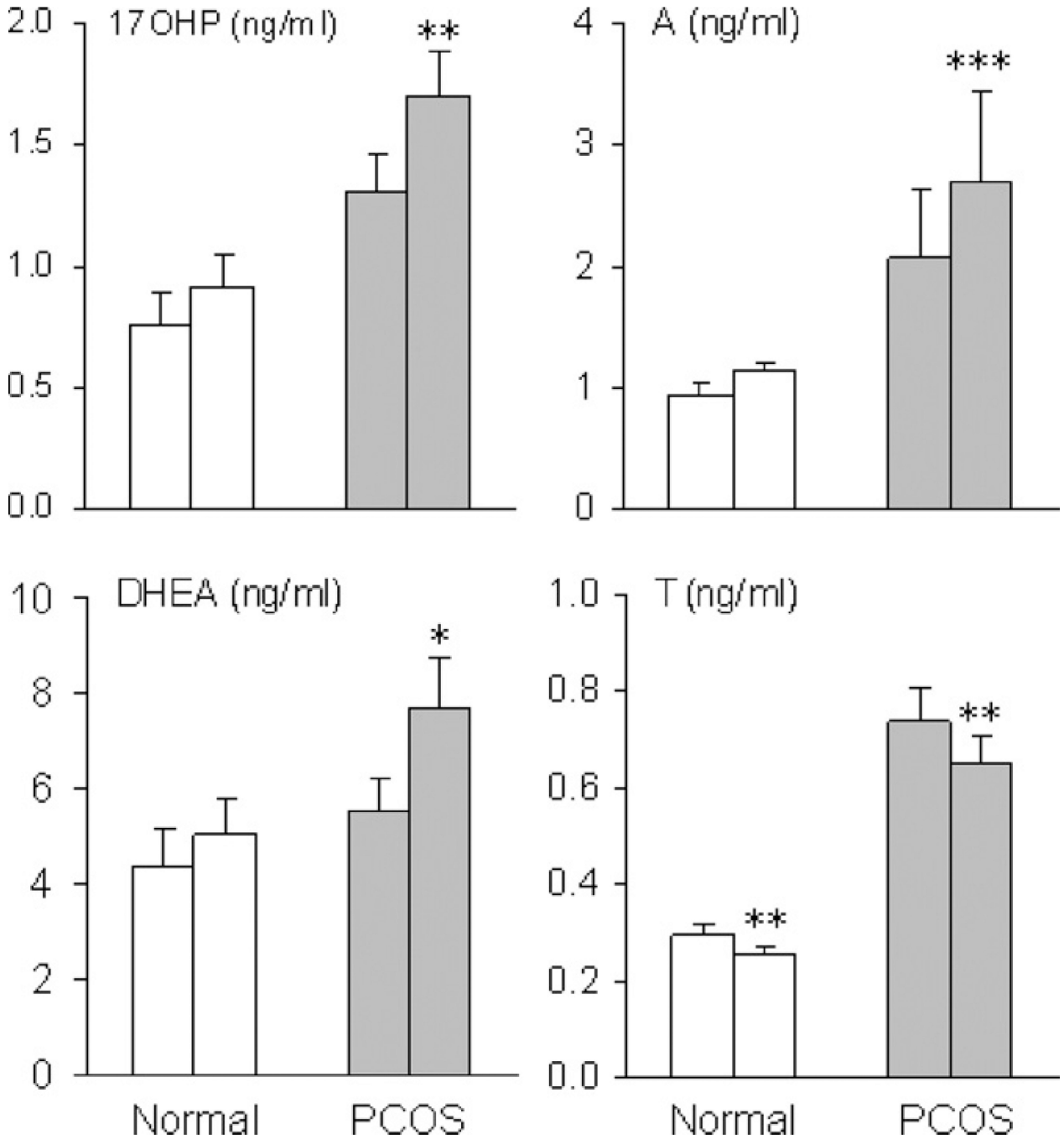

Fig. 7.

Mean (±SE) serum 17-OHP, A_4, DHEA, and T levels before and 24 h after administration of hFSH, 150 IU, in PCOS and normal women. A significant change from baseline is denoted by an *asterisk*. *$P < 0.05$; **$P < 0.02$; ***$P < 0.001$. (Conversion of 17-OHP, A_4, T, and DHEA to SI units by factors of 3.03, 3.49, 3.47, and 3.47, respectively).

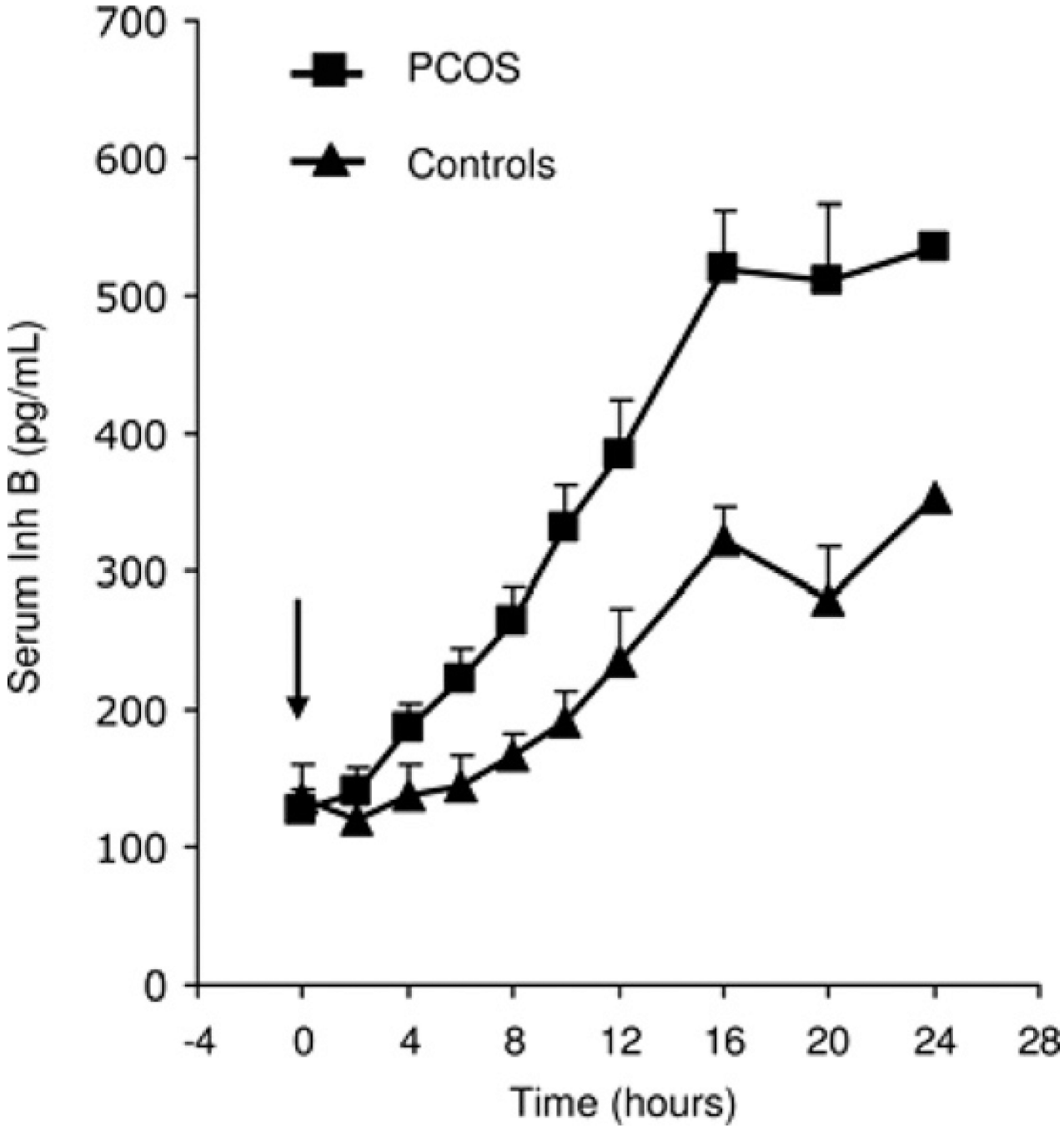

Fig. 8.
Mean (±SE) serum Inh B levels after administration of r-hFSH, 150 IU, in PCOS and normal women. Arrow indicates injection of FSH. (Conversion of Inh B to SI units by factor of 1).