Growth of the dominant follicle is similar to normal in patients with gonadotrophin-stimulated polycystic ovary syndrome exhibiting monofollicular development during a decremental dose regimen

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The aim of this study was to investigate the late follicular phase of seven gonadotrophin-treated patients with polycystic ovary syndrome (PCOS) exhibiting monofollicular growth and to compare developmental characteristics with the dominant follicle in seven regularly cycling control women. Daily serum follicle-stimulating hormone (FSH) levels in patients with PCOS decreased more rapidly compared to controls (−0.3 ± 0.2 IU/day in controls versus −0.7 ± 0.4 IU/day in PCOS; p < 0.02). No statistically significant differences were seen in daily increase (30% in controls and PCOS) and mean peak levels (825 ± 94 pmol/l in controls versus 937 ± 231 pmol/l in PCOS) of oestradiol (E₂) serum levels when comparing both groups. Mean daily growth of the dominant follicle (1.7 ± 0.4 mm in controls versus 1.9 ± 0.6 mm in PCOS) was not significantly different. It is concluded from the present study that in patients with PCOS treated with gonadotrophin plus adjuvant gonadotrophin-releasing hormone agonist, development of a single follicle can occur using a decreasing dose regimen resulting in decreasing serum FSH levels. In addition, growth and oestrogen production by the dominant follicle in PCOS is not significantly different from follicle growth under normal conditions.

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Patients suffering from anovulatory infertility and polycystic ovary syndrome (PCOS) are frequently resistant to clomiphene citrate treatment and need gonadotrophins to induce ovulation (1). In these patients, arrested development of ovarian follicles appears to be due to disturbed selection of the dominant follicle. This concept of normal early follicle development is based on observations regarding aromatase activity of cultured granulosa cells (2), follicular fluid steroid hormone estimates (3) and careful monitoring of ovarian function using transvaginal sonography (4, 5). It therefore seems of special interest to explore further the developmental characteristics of follicles after selection has taken place in PCOS due to a transient elevation of serum follicle-stimulating hormone (FSH) concentrations subsequent to exogenous gonadotrophin administration.

Assessment of growth rates of large ovarian follicles during multiple follicle development in gonadotrophin-stimulated cycles is hampered by difficulties with recognizing individual follicles during successive sonographic investigations. Moreover—in sharp contrast to the late follicular phase during the normal menstrual cycle (6)—the correlation between serum oestradiol (E₂) levels and follicle size disappears if growth of multiple large follicles is observed under these conditions (7). The aim of this study was to investigate the late follicular phase of patients with PCOS exhibiting growth of one single dominant follicle following gonadotrophin induction of ovulation using a decreasing dose regimen and adjuvant gonadotrophin-releasing hormone (GnRH) agonist medication as described previously (8). Observations in regularly cycling women served as control.

Materials and methods

Subjects and study protocol

Seven (from a total group of 35) gonadotrophin-treated infertile patients (for 4.7 ± 1.5 (±SD) years) with PCOS were included from our infertility clinic, based on the presence of not more than one follicle of ≥12 mm diameter during gonadotrophin induction of ovulation. Three patients were amenorrheic and four were oligomenorrheic (cycle length 44.7 ± 5.1 days). Their mean age was 29.4 ± 3.2 years and their body mass index (BMI) was 29.1 ± 4.9 kg/m². They all failed to ovulate using up to 150 mg of clomiphene citrate daily for five consecutive days. Polycystic ovary syndrome was
defined according to strict clinical (BMI, Ferriman Gallwey score) and endocrine (elevated dehydroepiandrosterone sulphate (DHEAS)) serum levels ($\geq 10 \mu\text{mol/l}$) and high free androgen index (FAI = testosterone $\times 100$/sex hormone binding globulin (SHBG) $\geq 5$) criteria and the polycystic appearance of ovaries by transvaginal sonography, as published previously (9). Mean luteinizing hormone (LH) levels were 7.9 $\pm$ 2.1 IU/l, whereas FSH concentrations were 4.7 $\pm$ 1.4 IU/l. Sonographic and endocrine data of seven healthy regularly cycling women (mean age 28.0 $\pm$ 1.2 years, BMI 21.7 $\pm$ 2.3 kg/m$^2$ and cycle length 28 $\pm$ 2 days) recruited through advertisement served as control.

This study was approved by the Ethics Review Committee of the Erasmus University/Dijkzigt Hospital and informed consent was obtained from all participating women. In all patients with PCOS, gonadotrophin therapy was combined with and preceded by GnRH agonist (Buserelin; Hoechst, Amsterdam, The Netherlands) treatment (3 x 400 $\mu$g/day, intranasally). The GnRH agonist treatment was started on the first day of a spontaneous or progestagen withdrawal bleeding and was continued for a period of 3 weeks, followed by concomitant treatment with randomly chosen gonadotrophins – Humegon (Organon Int, Oss, The Netherlands; N = 4) or Metrodin (Serono, Amsterdam, The Netherlands; N = 3). Starting on the first day of gonadotrophin administration, daily blood withdrawal (just preceding gonadotrophin injections) and transvaginal sonography took place. Sonography was performed between 12.00 and 16.00 h by the same observer (DCS), according to previously validated methods (4). A 5-MHz transvaginal transducer (Model 1550; Philips Medical Systems, Eindhoven, The Netherlands) was used. Mean size of ovarian follicles (>2 mm diameter) was computed by measuring two or three dimensions (longitudinal, anteroposterior and transverse) depending on size.

The initial dose of gonadotrophins was 150 or 225 IU/day im. The dose was decreased by 0.5 or 1 A (=75 IU)/day if at least one follicle exceeded a diameter of 9 mm. A further decrease to 75 IU/day was continued until the administration of a human chorionic gonadotrophin (hCG) (Pregnyl; Organon Int). When the mean diameter of the dominant follicle exceeded 18 mm, Buserelin and the gonadotrophins were discontinued and 10,000 IU of hCG were administered. No luteal support was provided.

**Hormone assays**

After withdrawal, blood was centrifuged within 30 min and serum was stored at $-20^\circ$C until assayed. The serum FSH levels were determined using a commercially
available immunoradiometric assay kit (Medgenix; Fleurus, Belgium) as described previously (9). Data are expressed in terms of the MRC 78/549 reference preparation, and intra- and interassay coefficients of variation were less than 3% and 8%, respectively. Serum E₂ levels were estimated by radioimmunoassay (3). Intra- and interassay coefficients of variation were 5% and 8%, respectively.

Data analysis
Endocrine and sonographic data obtained on the last day before the spontaneous LH peak in controls were compared with data obtained on the day of hCG administration in patients with PCOS (estimated interval of 12 h between LH and ovulation versus that 36 h between hCG and ovulation). "Presumed selection" was defined, based on previous studies under normal conditions (4), as the day that one follicle could be visualized at a size exceeding a mean diameter of 10 mm. The minimum size of a dominant follicle measured during gonadotrophin induction of ovulation in patients with PCOS was decided to be 12 mm owing to the potential interference of one or more large (8–12 mm) secondary follicles. Results are presented as means ± sd, unless stated otherwise. Comparison of hormone patterns between the treatment group and controls was performed using Mann–Whitney's test and Repeated Measurements Analysis of Variance. Sonographic observations were compared using Mann–Whitney's test or Wilcoxon's test. The p values given are two-sided and 0.05 was considered to be the limit of statistical significance.

Results
On the day of presumed selection the mean FSH levels in the control group were 4.4 ± 0.8 IU/l, whereas the FSH concentrations in the PCOS group were 6.8 ± 2.3 IU/l (p = 0.3) (Fig. 1). The FSH levels on the day before the LH surge in controls were not significantly different from those in the PCOS group on the day of hCG administration (3.3 ± 1.8 IU/l in controls versus 3.4 ± 2.2 IU/l in PCOS). Daily serum FSH levels in patients with PCOS decreased more rapidly as compared to controls (−0.3 ± 0.2 IU/day in controls versus −0.7 ± 0.4 IU/ day in PCOS; p < 0.02). Mean FSH levels on the first day of gonadotrophin administration were not significantly different from those levels on the day of hCG administration in the PCOS group (2.9 ± 1.6 IU/l versus 3.4 ± 2.2 IU/l; p = 0.06).

Mean E₂ concentrations on the day of presumed selection in the treatment group were 255 ± 137 pmol/l compared to 211 ± 108 pmol/l in controls. No statistically significant differences were seen in daily increase (30% in controls and PCOS) and mean peak levels (825 ± 94 pmol/l in controls versus 937 ± 231 pmol/l in PCOS) of E₂ serum levels when comparing both groups.

Discussion
Based on previous studies (4), it is assumed that selection of the dominant ovarian follicle is disturbed in patients suffering from PCOS. Normal FSH serum levels in the patients (9) are insufficient to induce adequate aromatase activity (2, 3) (i.e. the FSH threshold for follicle stimulation is augmented). In these patients, follicles can be stimulated to ongoing development by a transient increase of FSH serum concentrations subsequent to exogenous administration of gonadotrophins. It has been demonstrated (8) that diminished stimulation of non-dominant follicles beyond the day of presumed selection by decreasing the daily dose of exogenous gonadotrophins reduces the number and size of functional medium-sized follicles. Moreover, follicles that have reached a certain stage of maturation will continue their development in the presence of decreasing serum FSH concentrations (8).

Comparison of previous studies concerning follicle development in gonadotrophin-induced cycles was hampered by differences in patient criteria, applied preparations and dose regimens (10, 11). In addition,
the definition of “monofollicular” growth in induced cycles revealed discrepancies, partially due to the transabdominal technique of sonography (12). Simultaneous development of secondary medium-sized follicles and differences in timing of hCG injections can explain differences in growth slopes, oestrogen production and preovulatory size of large follicles (13). Using pulsatile subcutaneous administration of FSH in a low-dose incremental dose regimen, follicle growth was also suggested to be similar to normal (14). In the present study exhibiting decreasing serum FSH concentrations—owing to the applied decreasing dose regimen—it was demonstrated that growth and oestrogen production by the dominant follicle is similar to that of the normal menstrual cycle. This finding is in agreement with animal studies (15) suggesting that the dominant follicle is less dependent on FSH stimulation. This may be due to introvarian auto- or paracrine upregulation (16). Although all gonadotrophin-treated patients exhibiting monofollicular growth selected for this study fulfilled the same strict criteria for definition of PCOS, it cannot be ruled out that this study includes only a specific proportion of all patients with PCOS.

It may be concluded from this preliminary study that if monofollicular development occurs in gonadotrophin-treated patients with PCOS using decreasing dose regimens (and/or GnRH agonist treatment), growth and steroidogenic activity of the dominant follicle is indistinguishable from normal. Once the formation of a dominant follicle has been induced by a transient increase in serum FSH, further development is normal despite increasing serum FSH levels. Serum FSH levels during the late follicular phase in gonadotrophin-treated patients with PCOS showed a return to the early follicular phase serum FSH concentration and were similar to normal preovulatory levels. This gives further support to the notion that in at least a proportion of patients with PCOS early follicle development is normal and only selection of the dominant follicle is disturbed.

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