Comparison of follicle steroidogenesis from normal and polycystic ovaries in women undergoing IVF: relationship between steroid concentrations, follicle size, oocyte quality and fecundability

M.P. Teissier1,2,3, H. Chable2, S. Paulhac1, Y. Aubard1

1Centre d’assistance médicale à la procréation, Service de gynécologie-Obstétrique; CHU Dupuytren, Limoges and 2Service de Biochimie et Biologie moléculaire, Faculté de Médecine, Limoges, France
3To whom correspondence should be addressed at: Centre d’assistance médicale à la procréation, Service de gynécologie-Obstétrique CHU Dupuytren, 2 Avenue Martin Luther King, 87042 Limoges Cedex, France.
E-mail: endocrinologie-limoges@unilim.fr

Studies of ovarian stimulation for IVF have suggested a relationship between follicle size and pregnancy rates. Furthermore, the follicular endocrine environment is correlated with oocyte quality. The aim of this study was to verify the relationship between follicular steroid content, follicle size, oocyte maturity and fertilization outcome in women with normal ovaries following recombinant human FSH (rhFSH). Secondly, this study was extended to women with polycystic ovarian syndrome (PCOS). Fifty-nine patients (31 normal, 28 PCOS) underwent conventional IVF with rhFSH induction. Follicular diameter was classified as small (8–13 mm) or large (>14 mm) and sex steroid content was analysed for each group. Oocyte maturity was studied according to nuclear maturation the day after fertilization.

In both ovulation groups, 17β-oestradiol and progesterone concentration were significantly higher in large follicles with meiotically competent oocytes compared with those containing meiotically incompetent oocytes. Testosterone levels were increased in PCOS follicles compared with normal patients, with no difference between corresponding sub-groups of follicles with meiotically competent oocytes. The relationship between follicle size and embryo development showed that 14 mm could be a threshold value following rhFSH induction in normal or PCOS women.

Keywords: follicle size/IVF/oocyte maturity/polycystic ovarian syndrome/steroidogenesis

Introduction

Previous literature has suggested that follicular endocrine environment is related to oocyte quality in women (MacNatty et al., 1979; Van Dessel et al., 1996). In addition, a correlation between follicle size and oocyte maturity has been shown (Nilsson et al., 1985; Simonetti et al., 1985; Nayudu et al., 1989; Bergh et al., 1998). A relationship between follicle size and pregnancy rates was also reported in women following IVF induction (Nilsson et al., 1985; Dubey et al., 1995; Bergh et al., 1998). However, no consensus has been reached concerning maturity and therefore fecundability of oocytes contained in small follicles (Dubey et al., 1995; Inaudi et al., 1995; Bergh et al., 1998). All data thus far reported were obtained from women with normal ovaries and from follicles collected under different ovarian conditions such as spontaneous cycles (Mason et al., 1994; Van Dessel et al., 1996) or stimulated cycles following several induction protocols (Ben-Rafael et al., 1987; Yding Andersen, 1990, 1993; Suchanek et al., 1994; Wittmaack et al., 1994; Enien et al., 1998). It was postulated that a relationship exists between follicular steroid content, follicular size and oocyte maturity which could influence oocyte fertilization outcome in normal patients. The aim of this study was to verify this concept following recombinant human FSH (rhFSH) treatment in follicles collected from women with normal ovaries during a conventional IVF programme because differences could be dependent on the induction treatment used (Enien et al., 1995). Few data have been reported concerning follicular content in normal patients following rhFSH therapy (Duijkers et al., 1997; Teissier et al., 1999).

To achieve this, sex steroidogenesis was analysed in normal ovaries and PCOS follicles which were divided into two size classes and studied individually in relationship to nuclear oocyte maturity and fertilization outcome. Additionally, this analysis tried to determine whether or not follicular size is a valid criterion for selecting meiotically competent oocytes (MCO), related to local hormonal status in follicles induced by rhFSH in patients with normal ovaries as well as in PCOS.

Materials and methods

Subjects

Women with normal ovaries (40 patients) and women with PCOS (35) undergoing IVF participated in this study. All patients either had a tubal factor or anovulatory infertility, without any endometriosis observed on laparoscopy. Male partners had normal semen quality according to World Health Organization (WHO, 1992) criteria. Before entering the IVF programme, PCOS (25 ± 4 years old) and normal ovulation patients (28 ± 4 years old) were classified according to menstrual history and paraclinical data. Hormonal analysis and ultrasound examination were performed during the early follicular

E-mail: endocrinologie-limoges@unilim.fr
phase (days 2–5) of a spontaneous cycle in order to determine the ovulation profiles.

The criteria used were clinical, hormonal parameters and ultrasound pelvic data, following the same methodology previously used (Teissier et al., 1999).

Clinical data
Data were recorded regarding spontaneous menstrual cycle duration (normal = 26–32 days), presence of ovulation, dysovulation or anovulation on thermic curves (at least 3), cutaneous hyperandrogenism signs, body mass index (BMI) weight (kg)/height$^2$ (m) (normal range: 20–25).

Hormonal parameters
LH:FSH (mU/ml) ratio (normal <2), prolactinaemia (normal <25 ng/ml), basal plasmic concentrations of testosterone (normal <1000 pg/ml), dehydroepiandrostenedione sulphate (DHEA-S) (normal <3000 ng/ml) and 17β-oestradiol (normal 40–80 pg/ml).

Ultrasound pelvic examination
Ovary size (normal volume <8 ml), presence of micropolycystic (2–8 mm diameter) formations around the cortex (at least 15) and/or stromal hypertrophia (Franks, 1989) were recorded.

PCOS was diagnosed when at least two abnormalities of these parameters were observed (Yen et al., 1993; Franks, 1989). Normal patients had none of these pathological criteria.

IVF protocol
All women followed a gonadotrophin-releasing hormone agonist (GnRHa) protocol. D-Trp$^6$ analogue (Decapeptyl 0.1$,^1$ IPSEN Biotech Laboratories, Paris, France) was begun on day 1 of the menstrual cycle, at a daily dose of 0.1 mg administered s.c. D-Trp$^6$ analogue was continued until the day of human chorionic gonadotrophin (HCG) administration. When pituitary-ovarian axis down-regulation was achieved rhFSH induction was started (Gonal-F®, Serono Laboratories, Boulougne, France). Daily preparations were administered at an initial dose of 150 to 225 IU rhFSH. This rhFSH dose was maintained or increased until an adequate serum oestradiol response was attained in agreement with ultrasound follicular growth monitoring. Then 10 000 IU HCG (HCG 5000 IU, Organon, Puteaux, France) were given when at least five follicles were present with an average follicular diameter >16 mm.

IVF procedures
Collection techniques and IVF
For this study, 150 oocytes and follicular fluids were individually collected for IVF by ultrasonographically-guided vaginal puncture, 35 h after HCG administration. For each woman, two follicular fluid samples were obtained from different size follicles according to the ultrasound diameter measured. Each sample was collected separately in a syringe without culture medium. For this reason it was preferred to limit this selective puncture to two samples for each woman in order to avoid potential consequences for IVF outcome. Follicular size (mm), assessed at the time of the puncture, was noted. Follicular fluids selected for the study were free of blood and contained only one oocyte. Abnormal oocytes (dark-colour, fragmented zona pellucida) were excluded.

Follicle classification
After exclusion, a total of 118 follicular fluids was studied. In order to analyse the relationship between size and maturity of oocytes contained in these follicles, sub-groups were defined according to the following criteria. First, follicles were classified by diameter, two size sub-groups were defined. Follicles were considered as large (L) when the diameter was >14 mm and small (S) when the diameter was between 8–13 mm. This size classification was based on previous data reported in the literature. Following induction, different authors define a follicle as ‘large’ using different criteria, i.e. >12 mm (Testart et al., 1991), >15 mm (Fowler et al., 1977), or >16 mm (Bergh et al., 1998). In agreement with Dubey et al. (1995), who classify as ‘small’ follicles with mean diameter <14 mm, it was considered that 14 mm was an interesting intermediate value according to these conflicting reports. After retrieval, oocytes were immediately removed from the follicle aspirates and inseminated 1 h later with 90 000 motile spermatozoa. Nuclear maturation of oocytes was used for grading maturity (Testart et al., 1989; Mahadevan and Fleetham, 1990), 17–24 h after conventional IVF. Oocytes were considered as meiotically competent (MCO) when at least one polar body was extruded and as meiotically incompetent (MIO) when no polar body was present. The follicle population was divided, according to oocyte nuclear maturity, into the following functional classes: class A, follicles containing MCO and class B, other follicles.

Embryo development was noted for each oocyte, 48 h after fertilization.

Finally for the comparative study, follicular fluids were studied according to the following criteria: follicular size and oocyte nuclear maturity (four categories) and fertilization outcome in each ovulation group (PCOS or normal ovary patients).

Steroid assays
Follicular fluid was analysed individually because measurements on pooled specimens could modify the interpretation of results. Radioimmunoassay kits were used as previously described (Teissier et al., 1999). All assays were performed separately at least twice and the mean values were used for statistical analysis. Steroid analysis included: 17β-oestradiol concentrations determined with Coat-a-Count Estradiol kit (Berthing, Diagnostic Products Corporation, Los Angeles, CA, USA); testosterone concentrations quantified with Dria-Testok kit (Sorin Biomedica Diagnostics, Saluggia, Italy) and progesterone using the Gamma Coat [125I-] Progesterone CA 1724 kit (Incstar Corporation, Stillwater, MN, USA). Intra- and inter-assay coefficients of variation were 4.5–5, 6.5–7.8 and 6.9–10.1% respectively.

Statistical analysis
Steroid concentrations are presented as median and range because data were not normally distributed leading to the use of non parametric tests. Comparisons of steroid content between two groups were performed using the Mann-Whitney U test. Linear correlation analysis (Pearson rank) was used to identify correlations in the parameters tested. Contingency analyses by least square ($\chi^2$) or Fisher’s test, were performed to compare qualitative data. Significance was assumed at $P < 0.05$. Follicular hormone data are shown as box-and-whisker plots. The upper and lower edges of the box indicate the 25th and 75th percentile respectively, whereas the median is shown in the box. The whiskers represent minimum and maximum values. This part of the statistical analysis was performed using Statview 4.5 software (Abacus Concept Inc., Berkeley, CA, USA). Moreover, a cluster analysis was used in order to perform a similarity study (Fowler et al., 1977), looking for regroupment of steroid values in homogenous sub-groups from both populations. In agreement with Ward’s methodology (Everitt, 1989) a hierarchical cluster analysis was performed using average linkage between steroid content from the different follicle sub-groups previously described according to follicle size, oocyte maturity and fertilization outcome using Statistics Package for Social Sciences version 6.01 (SPSS Inc., Chicago, IL, USA).
Table I. Relationship between follicular steroid concentrations and follicle size from PCOS and normal women

<table>
<thead>
<tr>
<th></th>
<th>PCOS</th>
<th></th>
<th>Normal</th>
<th></th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small (n = 13)</td>
<td>Large (n = 43)</td>
<td></td>
<td>Small (n = 17)</td>
<td>Large (n = 45)</td>
</tr>
<tr>
<td>17β-oestradiol (ng/ml)</td>
<td>345 (197–762)</td>
<td>371 (189–1138)</td>
<td>NS</td>
<td>348 (131–1195)</td>
<td>393 (102–1310)</td>
</tr>
<tr>
<td>Testosterone (ng/ml)</td>
<td>7.2 (3.7–25.3)</td>
<td>7.9 (3.7–35)</td>
<td>NS</td>
<td>5.8 (2.6–13.8)</td>
<td>6.2 (1.7–23)</td>
</tr>
<tr>
<td>Progesterone (ng/ml)</td>
<td>4204 (2116–6247)</td>
<td>5363 (2127–8448)</td>
<td>NS</td>
<td>3470 (1086–9828)</td>
<td>5749 (2116–14484)</td>
</tr>
</tbody>
</table>

Results are expressed as median and (range).

n = number of subjects; PCOS = polycystic ovarian syndrome; Normal = women with normal ovary endocrine profile; P is assessed using Mann–Whitney test; NS = not significant.

Table II. Steroid concentrations from follicles according to nuclear oocyte maturity in both ovulation groups

<table>
<thead>
<tr>
<th></th>
<th>Class A (MCO-F) (n = 80)</th>
<th>Class B (MIO-F) (n = 38)</th>
<th>Class B versus class A</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PCOS</td>
<td>Normal</td>
<td>P</td>
</tr>
<tr>
<td>E2 (ng/ml)</td>
<td>473 (189–1138)</td>
<td>399 (211–1310)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(197–25.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>T (ng/ml)</td>
<td>6.3 (3.7–26)</td>
<td>5.8 (2.7–15)</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>(3.7–484)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pr (ng/ml)</td>
<td>5961 (4199–14484)</td>
<td>5951 (3054–9828)</td>
<td>NS</td>
</tr>
</tbody>
</table>

Results are expressed as median and (range).

Class A = follicles which contained meiotically competent oocytes (MCO-F); class B = follicles with meiotically incompetent oocytes (MIO-F); PCOS = polycystic ovarian syndrome; normal = women with normal ovary endocrine profile; E2 = 17β-oestradiol; T = testosterone; Pr = progesterone; P is assessed using Mann–Whitney test.

Results

Effect of follicular size on steroidogenesis
Steroid content was studied from 88 large and 30 small follicles (Table I). 17β-oestradiol and progesterone concentrations were correlated with follicle size (mm) (r = 0.35; P = 0.0004 and r = 0.39; P < 0.0001 respectively). Means comparison analysis showed significant differences between large and small follicles for 17β-oestradiol and progesterone concentrations (P < 0.003 and P < 0.0009 respectively). Adversely, no significant correlation was seen according to follicle diameter and testosterone concentrations.

When mean comparisons were performed for each ovulation group, these differences were only significant for 17β-oestradiol and progesterone concentrations in normally ovulating patients. No difference was seen in steroid concentrations from the PCOS group (Table I).

Relationship between steroidogenesis and oocyte quality
Sex steroid concentrations from follicles containing MCO (class A) differed significantly from class B follicles: for 17β-oestradiol, testosterone and progesterone concentrations P were respectively < 0.001, < 0.001 and 0.009.

Table II summarizes median steroid concentrations and P values following analysis of steroid concentrations comparing follicles according to oocyte quality, in each ovulation group.

When statistical analysis was performed on these sub-groups according to both ovulation profiles, no significant difference was seen for 17β-oestradiol, testosterone and progesterone concentrations from both class A follicles (Table II). In contrast, testosterone and progesterone concentrations were significantly higher in class B follicles from PCOS than from normal ovaries (Table II).

Moreover, significant differences were seen between class A and class B follicle steroid concentrations in each ovulation group (Table II).

Relationship between follicular size and oocyte quality
A discrepancy was found between size and maturity status of oocytes collected in both ovarian endocrine profiles. Some mature oocytes (MCO) were present in small follicles and immature ones (MIO) in large follicles, in each ovulation group. In PCOS patients four mature oocytes were found among the 13 small follicles and 33 in the large class. Similarly, 10 PCOS large follicles also contained MIO among the 43 large follicles studied. The same tendency was observed in normal patients: 35 MCO were seen among the large and eight among the small follicles; nine MIO (class B) were seen from the small follicles and 10 among the largest.

According to nuclear oocyte maturity and size criteria, contingency analysis was significant when whole data were
considered ($n = 118$ follicles), without taking into account the ovulation group ($\chi^2 = 46.1; P < 0.0001$). The same finding was observed for PCOS and normal ovulation patient groups ($\chi^2 = 29.7; P = 0.0001$ and $\chi^2 = 20.9; P < 0.0001$ respectively). In fact, most of class A follicles were retrieved from the large size group (33 from PCOS and 35 from normal).

### Relationship between steroid concentrations, follicle size and oocyte quality

Table III compares steroid concentrations between PCOS and normal patients, from follicles classified in the four sub-groups according to size and oocyte nuclear maturity. $17\beta$-oestradiol and progesterone concentrations tended to be higher in large follicles which contained MCO in each ovulation group (class A), without any difference between PCOS and normal patients (Table III). Significant differences existed only between large class A and large class B follicles from normal as well as from PCOS patients in $17\beta$-oestradiol concentrations (Table III). Testosterone concentrations were significantly lower in class A than in class B in both large and small follicles in the PCOS group (Table III). A significant difference was only seen between small class A and small class B follicles, in the normal ovulation group (Table III). For both PCOS and normal groups, progesterone concentrations were significantly higher in class A compared to class B follicles regardless of size (Table III).

No significant difference was seen for any steroid when a mean comparison was performed between the corresponding sub-groups from normal ovulation and PCOS class A follicles with MCO (data not shown).

### Relationship between steroid concentrations, follicle size, oocyte quality and fertilization outcome

Median values and range of steroid concentrations assessed in sub-group of follicles according to fertilization outcome are shown in Figure 1.

The relationship between follicle size, nuclear oocyte maturity and fertilization outcome was also studied in Table IV. Contingency analysis according to follicle size class and fertilization rate showed that embryo development was present in the largest follicles ($\chi^2 = 17.9; P = 0.0001$). The same results were found for each ovulation group (for normal ovulation $\chi^2 = 9.6; P = 0.002$ and for PCOS, $\chi^2 = 10.7; P = 0.006$).

Cluster analysis confirmed the correlation between steroid concentrations, follicle size, oocyte quality and fertilization outcome into both groups of patients. Cluster linkage showed that normal large follicles containing MCO were statistically grouped with the corresponding class from PCOS group (data not shown).

Comparison of mean steroid concentrations showed only a significant difference between small (<14 mm) and large follicles which contained oocytes leading to embryo development, with $P < 0.001$ for each steroid (Figure 1). No difference existed in steroid concentrations between either size of follicles containing oocytes which failed to develop (Figure 1).

### Discussion

Following rhFSH induction, the present study reports a correlation between follicular size and local steroidogenesis in normal patients (Table I). This has already been suggested using other induction protocols (Ben Rafael et al., 1987; Yding Andersen et al., 1990, 1993; Suchanek et al., 1994, Enien et al., 1998). However, this study emphasizes that follicle size alone is not a criterion related to local steroidogenesis in PCOS patients.

According to nuclear maturity of oocytes, in-vivo $17\beta$-oestradiol concentrations were higher in class A (with MCO) than in class B of follicles from each group of patients, suggesting that $17\beta$-oestradiol follicle concentration, following rhFSH induction, is correlated with oocyte quality regardless of the endocrine profiles of women (Table II). This finding has been previously reported in spontaneous cycles (McNatty et al., 1979; Mason et al., 1994; Van Dessel et al., 1996)

---

### Table III. Comparison of steroid concentrations between PCOS and normal women according to oocyte quality and follicle size

| Size | PCOS | | | Normal | | |
|------|------|------|------|------|------|
|      | class A ($n = 37$) | class B ($n = 19$) | A versus B $P$ | class A ($n = 43$) | class B ($n = 19$) | A versus B $P$ |
| $E_2$ (ng/ml) | | | | | | |
| S | 426 (242–763) | 325 (197–477) | NS | 363 (211–392) | 347 (131–395) | NS |
| L | 503 (189–1138) | 310 (190–471) | 0.001 | 412 (220–1310) | 286 (102–356) | 0.001 |
| $T$ (ng/ml) | | | | | | |
| S | 6.2 (3.7–17) | 7.9 (4.8–25) | 0.05 | 4.4 (2.7–8) | 9.8 (3.1–13.8) | 0.02 |
| L | 6.6 (3.7–26) | 10.4 (4.9–35) | 0.02 | 6.2 | 5 | NS |
| $Pr$ (ng/ml) | | | | | | |
| S | 5870 (4204–8448) | 3290 (2127–6078) | 0.009 | 5114 (3440–6247) | 2481 (915–3501) | 0.001 |
| L | 6622 (4199–14484) | 2791 (2116–4143) | 0.001 | 6567 (3054–9828) | 2036 (1086–3916) | 0.001 |

Results are expressed as median (range).

PCOS = polycystic ovary syndrome; Normal = women with normal ovary endocrine profile; $E_2 = 17\beta$-oestradiol; $T = \text{testosterone}; Pr = \text{progesterone},$

class A = follicles which contained meiotically competent oocyte; class B = follicles with meiotically incompetent oocyte; $S = \text{small follicle (8–13 mm)}; L = \text{large follicle (>14 mm)}; P$ is assessed by Mann-Whitney test.
Follicular steroids, follicle size and oocyte maturity

![Figure 1. Box-and-whisker plots showing steroid concentrations from follicles according to size and fertilization outcome. (A) 17β-oestradiol, (B) Testosterone (C) Progesterone concentrations. Emb.neg. = oocytes which failed to develop into embryos. Statistical analysis was performed using the Mann-Whitney test. Significant differences exist only between (S) small and (L) large follicles containing oocytes leading to embryo development (Emb. pos.). See Results section.

but contested by other groups following human menopausal gonadotrophin (HMG) ovarian induction (Ben-Rafael et al., 1987; Inaudi et al., 1995). These differences in intra-follicular oestradiol concentrations may depend upon the type of induction protocol used (Enien et al., 1995).

However, in both ovulation groups, 17 β-oestradiol concentrations were significantly higher in large follicles containing MCO (class A) than in class B ones, compared with 17 β-oestradiol concentrations in small corresponding classes (Table III). These data have been also reported from HMG stimulated normal ovulation patients (Andreani et al., 1997). This result is in agreement with the significant positive correlation observed between 17 β-oestradiol production and increasing follicle diameter and confirms the data obtained from normal follicles induced following HMG administration (Inaudi et al., 1995). Undoubtedly healthy large follicles contained numerous competent granulosa cells compared with small ones, leading to high 17 β-oestradiol concentrations with no difference between ovulation groups. This differs strongly from steroidogenesis reports concerning unstimulated human follicles from women with a normal ovarian profile (Brailly et al., 1981; Adashi, 1994; Branisteau et al., 1997) or PCOS (Agarwal et al., 1996). These findings suggest that rhFSH may regulate aromatase activity by acting on its expression by granulosa cells in large follicles, from normal as well as from PCOS ovaries, during induction (Erickson et al., 1992; Mason et al., 1994; Yong et al., 1994; Agarwal et al., 1996). The results in the current study also suggest differences in local physiological regulation of follicular growth and oocyte maturation between ovarian profiles for small follicles. It is postulated that, in IVF ovarian induction up to follicle retrieval, oocyte maturity and follicular growth are not closely related and can be independent of 17 β-oestradiol production below a threshold value of mean follicle diameter, in either ovulation profile (Salha et al., 1998) (Table III).

According to fertilization outcome, the 17 β-oestradiol concentrations (Figure 1) are in agreement with previous studies from normal patients (Tarlatzis et al., 1993; Suchanek et al., 1994) and in contrast to other reports (Ben-Rafael, 1987; De Geyter et al., 1992). This finding highlights that following rhFSH, local increasing oestrogen biosynthesis seems to be an important parameter for adequate human follicle and oocyte maturation leading to embryo development (Yding Andersen, 1993) in both ovulation groups.

Follicular testosterone concentration was significantly increased in total PCOS follicles compared with normal patients (P = 0.03). Size alone did not influence local testosterone production in either groups. These findings are in agreement with previous data concerning unstimulated cycles in PCOS women (Brailly et al., 1981). Differences in testosterone concentrations were found between both ovulation groups according to oocyte quality (Table II). But when follicle size and oocyte maturity were considered (Table III), no difference was seen between class A follicles with MCO in either group. Such findings agree with previously published studies (Erickson et al., 1992; Gilling-Smith et al., 1994; Hillier, 1997). In accordance with Brzynski et al. (1995), the results of the current study agree with the concept that excessive follicular androgen concentrations may affect oocyte quality (Brzynski et al., 1995). It could be concluded that follicular testosterone concentration differs according to oocyte maturity (Tables II, III) rather than follicle size (Table I) or ovulation profile (Table II).

According to size criteria alone progesterone concentrations did not differ but progesterone biosynthesis was significantly increased in class A compared with class B follicles, in each ovulation group and for each size class (Tables I, III). These findings have also been previously reported (Inaudi et al.,
1995; Andreani et al., 1997) following HMG therapy. In contrast to the findings of Doldi et al. (1998) no significant difference was seen in follicular progesterone production in the corresponding classes of follicles between PCOS and normal ovulation groups (Doldi et al., 1998). During rhFSH induction, it was observed that progesterone production seemed to be strongly correlated with the functional status (oocyte quality) rather than follicle size but not with the ovulation profile (Table III). This steroid concentration could be a better predictive factor for follicular growth and oocyte development (Hsu and Hsueh, 1997).

Finally, steroidogenesis seems to be similar in both ovulation groups according to follicle size, oocyte quality, and fertilization outcome under rhFSH induction in our IVF programme. These results are in agreement with recent clinical data following rhFSH induction in normal patients (Bergh et al., 1998; Out et al., 1999).

Clinically, follicle size is a classical parameter used for induction because it is objective and easily obtained for individual follicular evaluation (Nilsson et al., 1985). Previously, analysis of fertilization rate for all oocytes revealed a positive linear correlation with increasing follicle diameter (Dubey et al., 1995). In Dubey’s study 14 and 15 mm were assigned to the small class (Dubey et al., 1995). Follicles <14 mm have significantly lower rates of fertilization (Dubey et al., 1995). The results of the current study showed that following rhFSH induction, a follicle with diameter >13 mm could contain one oocyte with a correct nuclear maturity leading to embryo development in both ovulation groups (Table IV). This finding was also in agreement with those of Salha et al. (1998) and Penzias et al. (1994) which, however, point out the limits of sonographically measured mean diameters of follicles (Penzias et al., 1994; Salha et al., 1998). The previously classical value of 16 mm as determinant (Ectors et al., 1997; Out et al., 1999) could also be revised when considering rhFSH induction for normal and PCOS patients (El Sheikh et al., 1999) in conventional IVF cycles. The fertilization outcome (Figure 1, Table IV) obtained for follicles with a mean diameter between 13 and 15 mm, suggests that 14 mm might be considered an important criterion as described in PCOS (El Sheikh et al., 1999) as well as in control groups (Dubey et al., 1995). Finally, follicles between 14 and 16 mm could also be considered when selective puncture is used (i.e. patients do not want spare embryos to be frozen) (Salha et al., 1998). The possibility of evaluating oocyte maturity prior to fertilization is an advantage (Bergh et al., 1998) but this is not easy to do in conventional IVF. The study of the follicular endocrine micro-environment showed that follicle quality was related to size, nuclear oocyte maturity and oocyte viability (Figure 1) in normal as well as in PCOS ovaries following rhFSH (data not shown). This is in agreement with follicular fluid characteristics in normal ovulation patients resulting in successful implantation (Yding Andersen et al., 1993).

In conclusion, it is emphasized that following GnRHa plus rhFSH induction, healthy large follicles from PCOS patients can produce adequate follicular oestradiol and progesterone concentrations, in the same manner as normal patients. Analysis between local endocrine milieu, follicular size, nuclear oocyte maturity and fertilization outcome analysis tend to indicate that a 14 mm mean follicle diameter is a threshold value to predict oocyte fecundability with rhFSH induction in a standard long IVF protocol in normal as well as in PCOS women.

Acknowledgements

We thank Professor M.Rigaud for his help and Dr J.Cook for assistance in the preparation of the manuscript. We are also grateful to Drs P.Piver and A.M.Chinchilla for their participation in follicular fluid collection. This work was supported by a grant from Serono Laboratories Paris, France.

References


Received on March 8, 2000; accepted on September 8, 2000

Follicular steroids, follicle size and oocyte maturity


Received on March 8, 2000; accepted on September 8, 2000

2477