Prolonged HCG action affects angiogenic substances and improves follicular maturation, oocyte quality and fertilization competence in patients with polycystic ovarian syndrome

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BACKGROUND: The aim of this study was to determine whether, in polycystic ovarian syndrome (PCOS) patients, HCG action prolonged for 4 h improves the action of angiogenic substances [ovarian renin angiotensin system and vascular endothelial growth factor (VEGF)], and consequently follicular maturation, oocyte quality and oocyte fertilization competence. METHODS: In this prospective study 20 patients with PCOS undergoing IVF were included. Oocyte retrieval was carried out either 34 or 38 h after HCG administration. Each follicle was analysed for prorenin, active renin, VEGF and estradiol. Oocytes were evaluated for quality (mature, immature, degenerated oocytes), as were the embryos (low or high). RESULTS: In the HCG + 38 h group there were 245 follicles, and in the HCG + 34 h group 240 follicles. In the HCG + 38 h group, log active renin was lower (2.78 ± 0.20 versus 2.91 ± 0.25; P < 0.001) and VEGF higher (2276.0 ± 790.1 versus 1946.6 ± 954.5 pg/ml; P < 0.001). The odds ratio for obtaining oocytes from follicles was 1.6 [95% confidence interval (CI) 1.1–2.6; P = 0.02], and for developing high quality embryos 7.6 (95% CI 2.8–20.9; P < 0.001) in favour of the HCG + 38 h group. CONCLUSIONS: Follicular maturation and oocyte quality are related to the intrafollicular influences of active renin and VEGF in a time-dependent manner after HCG administration, whereas fertilization competence is related to VEGF only.

Key words: oocyte fertilization competence/oocyte quality/polycystic ovarian syndrome/renin angiotensin system/vascular endothelial growth factor

Introduction

Polycystic ovarian syndrome (PCOS) is a frequent condition, its incidence varying from 4% (Knochenhauer et al., 1998) to 22%, when the diagnosis is based on ultrasound findings only (Cresswell et al., 1997). It is manifested by a typical B-mode ultrasound morphological appearance of the ovary (Adams et al., 1985) associated with menstrual disorders (oligo- or amenorrhoea) and/or signs of hyperandrogenism (hirsutism, acne or alopecia) (Homburg, 2002). Frequently, elevated LH concentrations are also found in PCOS patients (Franks, 1989). In addition to increased androgen metabolism, the excess of LH may affect the syntheses of prorenin (Palumbo et al., 1993; Yoshimura, 1997) and vascular endothelial growth factor (VEGF) (Agrawal et al., 1998; Artini et al., 1998), which are angiogenic substances synthesized in the theca and granulosa cells (Glorioso et al., 1986; Agrawal et al., 2002), affecting angiogenesis (Yoshimura, 1997) in the follicular wall and in the corpus luteum (Van Blerkom et al., 1997; Ferrara, 2000).

After HCG administration, multiple follicular development increases the possibility of development of ovarian hyperstimulation syndrome (Schenker and Weinstein, 1978; MacDougall et al., 1993). Oocytes collected in PCOS stimulated cycles are often immature, of lower quality and have lower fertilization competence (Homburg et al., 1993; Aboulghar et al., 1997). The exact mechanism of this increased sensitivity of PCOS patients to exogenous gonadotrophins and HCG is not clearly understood, and little is known about the maturation and fertilization competence of oocytes or their developmental potential.

It seems that the ovarian renin angiotensin system (RAS) and VEGF have an impact on developmental and fertilization competence of human oocytes (Itskovitz et al., 1991; Van Blerkom et al., 1997; Loret de Mola et al., 1999). Prorenin is probably activated to active renin in the follicular fluid, where it can then complete the RAS cascade to angiotensin II (Ang II) (Culler et al., 1986; Daud et al., 1990), which has an impact on ovulation (Pellicer et al., 1988;
Kou et al., 1991). Owing to its short half-life, Ang II is difficult to measure accurately; therefore, active renin may serve as a sufficiently stable marker of Ang II activity (Peach, 1977).

It is known that VEGF stimulates the maturation of bovine oocytes in vitro, resulting in promotion of fertilization rates and subsequent development of embryos (Luo et al., 2002a). VEGF has a beneficial effect on the initial development of bovine embryo through surrounding cumulus cells (Luo et al., 2002b). The concentration of VEGF in the follicular fluid has been found to be five-fold higher in preovulatory follicles compared with early antral follicles (Einspanier et al., 1999).

The rates of first body formation and fertilization of oocytes matured in vitro are generally lower than in oocytes matured in vivo (Mikkelsen and Lindenberg, 2001; Trounson et al., 2001; Lin et al., 2003; Balakier et al., 2004).

The aim of this study was to investigate how follicular maturation, oocyte quality and fertilization competence are related to the intrafollicular influences of angiogenic substances, such as RAS substances and VEGF, in a time-dependent manner after HCG administration. We designed this study to investigate the effect of postponing follicular aspiration in PCOS patients for some hours (to 38 h after HCG administration, beyond the usual 34–36 h) to prolong the time of actions of RAS substances and VEGF to thus improve follicular maturation, oocyte quality and oocyte fertilization competence.

Materials and methods
In this prospective study, 20 patients with PCOS undergoing IVF were included. In 10 patients conventional IVF was carried out, and ICSI in 10 patients. The inclusion criteria were at least one of the typical symptoms present (menstrual disorders, hirsutism, acne, anovulatory infertility) and typical B-mode ultrasound scan (more than eight follicles of <10 mm in diameter in one plane of the ovary, usually in the subcapsular region, and enlarged, hyperechogenic central stroma) (Adams et al., 1985).

In all the patients ovarian stimulation was performed using GnRH analogues (Suprefact; Hoechst AG, Frankfurt/Main, Germany) administered from day 22 of the cycle in a daily dose of 0.6 ml (600 pg) subcutaneously. After 14 days, pituitary desensitization was started. GnRH analogue administration was continued until HCG (Primogonyl; Schering AG, Berlin, Germany) for oocyte maturation in a dose of 10000 IU was administered. HCG was administered when three or more follicles reached a diameter of ≥18 mm.

Twenty patients were allotted to two groups: in nine PCOS patients, oocytes were retrieved 34 h after HCG administration; in 11 PCOS patients, oocyte retrieval was postponed for 4 h.

Follicular fluid was aspirated separately from each follicle to self-aspirate during ultrasound-guided transvaginal oocyte retrieval.

We routinely use the aspiration needle set TIK (TIK d.d., Capistrano, CA, USA). Two different monoclonal antibodies to VEGF were used. One antibody was coupled to biotin and the other was radiolabelled for detection. The radioimmunoassay (RIA) that uses two monoclonal antibodies; one antibody was coupled to biotin and the other was radiolabelled for detection. The radiolabelled antibody only recognizes the active form of renin. By a slight modification of this assay, addition of 25 μl of a specific renin inhibitor, it was possible to measure total renin.

Total renin was measured by an assay (Nichols Institute Diagnostics) that uses two monoclonal antibodies; one antibody was coupled to biotin and the other was radiolabelled for detection. The radiolabelled antibody only recognizes the active form of renin. By a slight modification of this assay, addition of 25 μl of a specific renin inhibitor, it was possible to measure total renin.

Prorenin concentration was calculated by subtraction of active renin from the total renin concentration.

VEGF was measured by enzyme-linked immunosorbent assay kit (BioSource International, Camarillo, CA, USA).
The national medical ethics committee approved the study; all women signed an informed consent form before participation in the study.

Statistical analyses were performed using SPSS for Windows (SPSS, Inc., Chicago, IL, USA). Student’s t-test and Mann–Whitney U-test were used to compare normally and non-normally distributed variables, respectively. For multiple comparisons, Bonferroni correction was applied. The quality of oocytes and the quality of embryos were analysed by χ²-square test. Active renin and prorenin concentrations were log-transformed to allow the use of parametric tests. Differences were considered significant when P-values were < 0.05.

Results
The study population consisted of 485 follicles from 20 PCOS patients. The women’s mean age was 30.9 ± 3.1 years, mean body mass index was 23.7 ± 4.5 kg/m² and the mean number of ampoules of gonadotrophins used to achieve ovarian stimulation was 29.5 ± 6.0. Pregnancy was achieved in six (30%) women.

In the late oocyte retrieval group, 245 follicles were aspirated 38 h after HCG administration (HCG+38 h), and in the early oocyte retrieval group 240 follicles were aspirated 34 h after HCG administration (HCG+34 h). All the follicles with an estimated diameter of ≥ 14 mm were retrieved.

The distribution of normal-appearing MII, immature and degenerated oocytes was about the same in both groups. However, in the HCG+38 h oocyte retrieval group there were significantly fewer empty follicles (P = 0.03) (Table I).

The odds ratio for the total number of oocytes retrieved regardless of their quality was 1.6 [95% confidence interval (CI) 1.1–2.6; P = 0.02] in favour of the HCG+38 h oocyte retrieval group.

The assessments of fertilization and embryo quality were carried out separately for follicular aspirates after conventional IVF, and for those after ICSI.

Fertilization and embryo quality after IVF showed that there were more fertilized oocytes (P < 0.001) and more embryos of high quality (P < 0.05) in the HCG+38 h oocyte retrieval group (Table I).

Table I. Comparison of different quality oocytes, fertilization competence and high-quality embryo rates between two groups of PCOS patients

<table>
<thead>
<tr>
<th>Description</th>
<th>HCG +38 h (%)</th>
<th>HCG +34 h (%)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Empty follicular aspirates</td>
<td>128 (52.2)</td>
<td>149 (62.1)</td>
<td>0.029</td>
</tr>
<tr>
<td>Degenerative oocytes</td>
<td>16 (13.7)</td>
<td>10 (11.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Immature oocytes</td>
<td>7 (6.0)</td>
<td>5 (5.5)</td>
<td>NS</td>
</tr>
<tr>
<td>Normal-appearing MII oocytes</td>
<td>94 (80.3)</td>
<td>76 (83.5)</td>
<td>NS</td>
</tr>
<tr>
<td>IVF</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized oocytes</td>
<td>27 (81.8)</td>
<td>26 (42.7)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>High-quality embryos</td>
<td>20 (74.1)</td>
<td>12 (46.2)</td>
<td>0.038</td>
</tr>
<tr>
<td>ICSI</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fertilized oocytes</td>
<td>36 (42.9)</td>
<td>13 (43.3)</td>
<td>NS</td>
</tr>
<tr>
<td>High-quality embryos</td>
<td>21 (58.3)</td>
<td>3 (23.1)</td>
<td>0.029</td>
</tr>
</tbody>
</table>

Table II. Comparison of concentrations of prorenin, active renin, VEGF and E₂ in follicular aspirates (empty and those containing oocytes), and of follicular diameters between two groups of PCOS patients

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HCG +38 h (μ)</th>
<th>HCG +34 h (μ)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log of prorenin</td>
<td>4.09 ± 0.27 (209)</td>
<td>4.16 ± 0.29 (192)</td>
<td>0.01</td>
</tr>
<tr>
<td>Log of active</td>
<td>2.78 ± 0.20 (245)</td>
<td>2.91 ± 0.25 (240)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>VEGF (pg/ml)</td>
<td>2276.0 ± 790.1 (245)</td>
<td>1946.6 ± 954.5 (189)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>E₂ (nmol/l)</td>
<td>322.9 ± 49.2 (244)</td>
<td>340.9 ± 58.9 (238)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diameter of follicles (cm)</td>
<td>2.2 ± 0.4 (245)</td>
<td>2.1 ± 0.4 (240)</td>
<td>0.02</td>
</tr>
</tbody>
</table>

In the HCG+38 h group, oocyte retrieval was carried out 38 h after HCG administration; in the HCG+34 h group, it was done 34 h after HCG administration.

NS, not significant.

After ICSI, fertilization was lower owing to male factor infertility. However, the oocytes that were fertilized more frequently developed into high-quality embryos in the HCG+38 h group than in the HCG+34 h (P < 0.05) (Table I).

None of the immature oocytes and only two (7.7%) degenerated oocytes were fertilized.

Renin and VEGF concentrations in the follicular fluid regarding oocyte and embryo quality

Prorenin, active renin and E₂ concentrations were significantly lower, VEGF was significantly higher, and follicular diameters were significantly greater in the HCG+38 h oocyte retrieval group compared with the HCG+34 h oocyte retrieval group. All follicles, both empty and those containing oocytes, were considered for the analysis (Table II).

In addition, there were statistically significant differences in the concentrations of prorenin, active renin, E₂ and VEGF between the empty follicles in the HCG+38 h and those in the HCG+34 h group (prorenin: log 4.08 ± 0.25 versus log 4.18 ± 0.32, P < 0.001; active renin: log 2.78 ± 0.20 versus 2.94 ± 0.26, P = 0.008; E₂: 320.2 ± 49.6 versus 341.6 ± 64.2 nmol/l, P < 0.001; VEGF: 2251.0 ± 797.5 versus 1857.0 ± 881.1 pg/ml, P = 0.003).

Active renin and VEGF concentrations were found to be important in differentiating between the follicular aspirates containing oocytes and empty follicular aspirates. Active renin concentrations were statistically higher and VEGF concentrations were statistically lower in the empty follicular aspirates compared with the follicular aspirates containing normal-appearing MII oocytes (Table III). VEGF concentrations were statistically lower in follicular aspirates containing immature oocytes compared with follicular aspirates containing MII oocytes (Table III). Prorenin and E₂ concentrations did not differ between the empty follicular aspirates and the follicular aspirates containing oocytes of different quality (Table III).

Active renin concentrations of follicular aspirates containing normal-appearing MII oocytes were significantly different between the HCG+38 h and the HCG+34 h oocyte retrieval group. The difference in the VEGF concentration, however, was substantial, albeit not statistically significant (P = 0.055) (Table IV).

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In general, after conventional IVF, VEGF concentrations of follicular aspirates containing oocytes that were fertilized were higher (2238.8 ± 691 pg/l; n = 49) than of those containing oocytes that were not fertilized (1877.4 ± 901.6 pg/l; n = 32) (P = 0.045), regardless of the time of oocyte retrieval. On the other hand, log of active renin concentrations of follicular aspirates containing oocytes that were fertilized were lower (2.79 ± 0.19; n = 53) than of those containing oocytes that were not fertilized (2.86 ± 0.23; n = 41) (P = 0.077).

After conventional IVF, the odds ratio for normal-appearing MII oocytes that developed to high-quality embryos was 7.6 (95% CI 2.8–20.9; P < 0.001) in favour of the HCG + 38 h oocyte retrieval group.

Normal-appearing MII oocytes after IVF differed by follicular VEGF concentration, fertilization competence and embryo quality between the groups (Table V).

**Discussion**

Analysing the results of this study we found that in PCOS patients, HCG action prolonged for 4 h (prolonged time interval between HCG administration and oocyte retrieval extended from 34 h to 38 h) improves the expression and action of angiogenic substances such as RAS substances and VEGF, probably by acting on follicular vascularization, and consequently improves oocyte quality, fertilization competence and embryo developmental potential.

It is known that in PCOS patients the oocytes retrieved from stimulated cycles are often immature, and of low quality and low fertilization competence (Homburg et al., 1993). In addition, it is known that in-vitro matured oocytes have lower fertilization competence, lower embryo developmental potential and lower implantation rate compared with oocytes matured in vivo (Mikkelsen and Lindenberg, 2001; Chian, 2004).

In our study, we achieved improved follicular maturation and oocyte fertilization competence in PCOS patients in whom the time from HCG administration to oocyte retrieval was extended by 4 h. This is in agreement with Mansour et al. (1994), who found a significantly higher number of MII oocytes, fertilized oocytes and embryos after ICSI for severe male factor infertility in whom oocyte retrieval was performed 36 or 37 h after HCG administration than in the patients in whom oocyte retrieval was done 35 h after HCG administration.
In our study, there were 9.9% less empty follicles and 40.9% more embryos of good quality after conventional IVF in the HCG + 38 h oocyte retrieval group. Even after ICSI, the share of good quality embryos was 15% higher in the HCG + 38 h group. In the study by Mansour et al. (1994) involving normally cycling patients, the ratio of the number of MII oocytes to the total number of follicles was higher than in our study involving PCOS patients. Therefore, we believe that prolonging HCG administration is even more important in PCOS patients than in normally cycling patients.

Many factors are involved in follicular and oocyte maturation, fertilization and embryo development, one of them being optimally dissolved oxygen content in the follicle, which is provided by adequate follicular vascularization (Van Blerkom et al., 1997; Bhal et al., 1999). The final products of RAS, Ang II (Kuo et al., 1991) and VEGF (Agrawal et al., 1998; Artini et al., 1998), are the substances that have an important influence on angiogenesis and follicular vascularization, and consequently on follicular maturation and oocyte quality. Other known substances are leptin, interleukin (IL)-1, IL-6, IL-8, angiopoietin, insulin-like growth factor, basic fibroblast growth factor and endothelin-1 (Simon et al., 1998; Pellicer et al., 1999; Van Blerkom, 2000).

In our study population, active renin and VEGF were found to be important factors in differentiating the follicular aspirates containing MII oocytes from empty follicular aspirates.

As far as prorenin and active renin are concerned, we did not find any difference in follicular prorenin and active renin concentrations between mature and immature oocytes, which might be due to a high degree of scatter of prorenin and active renin levels among the follicles; however, a difference was found comparing active renin concentrations between follicular aspirates containing MII oocytes and follicular aspirates that were empty. Active renin was found to be a better predictor of follicular maturation than prorenin, which is in agreement with Hagemann (1997). We presume that the active renin concentration was low because it had already been consumed in the synthesis of Ang II, which affected follicular vascularization, and consequently follicular maturation.

It has been shown that the synthesis and discharge of Ang II is time-dependent on LH in unstimulated menstrual cycles or on HCG in stimulated cycles (Acosta et al., 2000). It has been demonstrated that there is an increase in Ang II concentrations in the ovarian venous plasma of the bovine ovary with mature follicles compared with the contralateral non-ovulating ovary 24–48 h after the peak of the LH surge. It was subsequently shown that this Ang II surge coincided with ovulation (Acosta et al., 2003).

Low follicular renin, found in the late oocyte retrieval group, indirectly indicates that prolonged HCG action has an effect on RAS substances, which have a beneficial effect on follicular maturation and oocyte quality. In this way we presumably achieved similar peri-ovulatory conditions to those observed in untreated natural cycles (Tokuyama et al., 2002).

In terms of VEGF concentrations, we demonstrated its role in follicular maturation, oocyte maturity and oocyte fertilization competence. The VEGF concentration was higher in the follicular aspirates containing MII oocytes in comparison with empty follicular aspirates. The VEGF concentration was higher also in follicular aspirates containing MII oocytes than in follicular aspirates containing immature oocytes, and in those oocytes that were fertilized compared with those that were not. This is in agreement with the report suggesting that VEGF is associated with high follicular vascularization and oxygenation, resulting in good-quality oocytes with superior fertilization competence (Van Blerkom et al., 1997).

Fertilization was lower after ICSI than after IVF owing to male factor infertility. However, if the oocytes were fertilized, male factor infertility was attenuated by the effect of VEGF, which was manifested through a higher number of high-quality embryos in the HCG + 38 h group. This has also been found in animal studies (Luo et al., 2002b).

In our previous studies (Vrtačnik Bokal and Meden Vrtovec, 1998; Vrtačnik Bokal et al., 2003) we found a higher vascular impedance to utero-ovarian blood flow in PCOS patients compared with normally cycling women in untreated natural cycles as well as in stimulated cycles, because of insufficient action of RAS substances on follicular vascularization. We assumed that with a prolonged follicular phase and prolonged time from HCG administration to oocyte retrieval, we could improve the follicular vascularization by prolonging the action of RAS substances and VEGF, in order to improve the follicular maturation, oocyte quality and fertilization competence.

In this study we confirmed our assumptions. A lower active renin concentration was found in the PCOS patients in whom the time from HCG administration to oocyte retrieval prolonged for 4 h. In this way, the binding capacity of active renin in the RAS cascade was augmented. This is necessary for the synthesis of Ang II and its action on follicular vascularization. Also, recent studies have demonstrated that Ang II upregulates VEGF locally (Richard et al., 2000; Tamarat et al., 2002). This was confirmed in our study by a better VEGF local follicular response due to increased RAS activity in the late oocyte retrieval group. According to these results, we assume that the final effect on folliculogenesis and oocyte fertilization competence is, among others (leptin, IL-1, IL-6, IL-8, angiopoietin, insulin-like growth factor, basic fibroblast growth factor and endothelin-1).
IL-8, angiopoietin, insulin-like growth factor, basic fibroblast growth factor and endothelin-1), also achieved by the synergic action of Ang II and VEGF (Simon et al., 1998; Pellicer et al., 1999; Van Blerkom, 2000).

In the literature, differences in follicular VEGF concentrations exist between untreated natural cycles and stimulated cycles. Higher follicular VEGF concentrations were found in untreated natural cycles than in gonadotrophin-stimulated cycles. In high responders, VEGF concentrations were lower than in low responders (Pellicer et al., 1999; Tokuyama et al., 2002). On the basis of these findings it was suggested that an increased number of mature follicles suppresses the production of VEGF in each follicle (Tokuyama et al., 2002).

In our high PCOS responder population we found higher VEGF concentrations in the HCG+38 h oocyte retrieval group than did Tokuyama et al. (2002). We suppose that the synthesis of VEGF in gonadotrophin-stimulated cycles is delayed because LH is suppressed, and after HCG administration more time is necessary for the VEGF synthesis to begin than in untreated spontaneous cycles. Another possible explanation might be that all follicles do not respond synchronously to HCG. It seems that with prolonged action of HCG a better VEGF expression is achieved in delayed follicles as well. In this way the conditions achieved are more similar to those in ovulatory untreated cycles with higher VEGF concentrations in the follicle.

Furthermore, normal-appearing MII oocytes from the follicles of similar size and with the same morphological characteristics differed in their fertilization competence. We found that fertilization and embryo developmental competence are determined by intrafollicular influences. Follicular aspirates containing oocytes that were fertilized had higher VEGF concentrations than follicular aspirates containing oocytes that were not fertilized. Significantly higher numbers of fertilized MII oocytes and high-quality embryos were found in the late oocyte retrieval group.

None of the immature oocytes was fertilized. In follicular aspirates containing immature oocytes, lower VEGF concentrations were found. This indicates the importance of VEGF in oocyte fertilization competence and embryo development in animal experiments (Luo et al., 2002a; b). VEGF administration induces balanced cytoplasmic and nuclear maturation in bovine oocytes, matured and fertilized in vitro (Luo et al., 2002b). We assume that in our study the same was obtained in vivo with prolonged action of HCG on the VEGF synthesis. This is also in agreement with the finding that VEGF released from cultured granulosa cells is time- and dose-dependent on HCG (Agrawal et al., 2002). It was found that MII oocytes which extruded the first polar body had better fertilization rates and embryo morphologies than in-vitro matured oocytes that extruded the first polar body following the removal of cumulus/corona cells in in-vitro culture (Huang et al., 2002; 2004). Also, it was shown that human oocytes progressively develop the ability for full activation and normal development during the MII arrest following the extrusion of the first polar body during in-vitro culture (Balakier et al., 2004).

We assume that the process of oocyte maturation in PCOS patients was not completed upon reaching the MII, and that maturation of normal-appearing MII oocytes was improved by prolonged action of HCG, which was manifested through follicular VEGF. Furthermore, VEGF has an impact on embryo developmental competence. In the late oocyte retrieval group, MII oocytes from follicular aspirates with higher VEGF concentrations developed to high-quality embryos in 67%, whereas in the early oocyte retrieval group only 21% of MII oocytes developed to high-quality embryos.

It is known that excessive LH-induced androgen excess in association with hyperinsulinaemia has detrimental effects on oocyte maturation, which is premature and abnormal in PCOS women. The retrieved oocytes have reduced fertilization competence and lower quality embryos (Homburg et al., 1993; Shoham et al., 1993). In our study we retrieved a small number of degenerated and immature oocytes, but a problem arose in normal-appearing MII oocytes, which had the same morphological characteristics but resulted in different oocyte fertilization competence and embryo development. We believe that we overcame the problem of equally appearing MII oocytes with different fertilization competence by HCG action prolonged for 4h.

We conclude that follicular maturation and oocyte quality are related to the intrafollicular influences of active renin and VEGF in a time-dependent manner after HCG administration, whereas fertilization competence is related to VEGF only. The prolonged HCG action on RAS substances and VEGF improves follicular maturation, oocyte quality and oocyte fertilization competence, and provides a higher number of high-quality embryos in PCOS women. The timing of oocyte retrieval after HCG administration seems to be a promising alternative to in-vitro maturation of oocytes that are devoid of normal intrafollicular environment.

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granulosa cells is higher in women with polycystic ovaries than in women with normal ovaries. Fertil Steril 78,1164–1169.


