Expression of angiotensin II type 1 (AT1) and angiotensin II type 2 (AT2) receptors in human granulosa-lutein (GL) cells: correlation with infertility diagnoses

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bCentro de Asistencia a la Reproducción Humana de Canarias, La Laguna, Tenerife, Spain; and cDepartment of Obstetrics and Gynecology, New York University School of Medicine, New York, New York

Objective: To correlate angiotensin II (AngII) receptor expression by granulosa-lutein (GL) cells from gonadotropin-stimulated follicles with infertility diagnosis and IVF parameters.

Design: The mRNA of angiotensin receptors type 1 (AT1) and type 2 (AT2) was studied in aspirated GL cells.

Setting: University laboratory and private IVF center.

Patient(s): Seventy-three IVF patients.

Intervention(s): Reverse-transcription polymerase chain reaction analysis for relative expression of AT1 and AT2 receptor mRNA in women with no ovarian factor (NOF), poor ovarian reserve (PR), endometriosis (ENDO), and polycystic ovary syndrome (PCOS).

Main Outcome Measure(s): Expression of AT1 and AT2 receptor mRNA.

Result(s): There was a constant ~7:1 ratio between AT1 and AT2 receptors and a negative correlation between the AT1/AT2 ratio and patient age. There were statistically significant differences in AngII receptors in individual conditions: NOF showed a correlation between AT1 and AT2 receptors and a negative correlation between AT1 receptor expression, embryo fragmentation and number of metaphase II (MII) oocytes; PR showed a negative correlation between AT2 receptor expression and number of MII oocytes; PCOS AT1 receptor expression correlated negatively with the units of FSH administered and with patients' age; ENDO showed no significant correlations.

Conclusion(s): Mural GL cells express AT1 receptor much more than AT2 receptor. AngII receptor expression varies with age and infertility diagnosis. Low expression of AngII receptors was associated with high-dose stimulation in women with PR. Embryo fragmentation in NOF is associated with decreased AT1 receptor expression, supporting a role for AngII in GL cell apoptosis. (Fertil Steril® 2010;93:1601–8. ©2010 by American Society for Reproductive Medicine.)

Key Words: Granulosa-lutein cells, in vitro fertilization, OVRAS, angiotensin II receptors, embryo fragmentation, oocyte maturation

In recent years, we and others have demonstrated that human and rat ovaries possess a complete and functional gonadotropin-sensitive renin-angiotensin system (OVRAS) (1–5). Follicular cells secrete and are affected by angiotensin II (AngII), triggering a diversity of effects that depends on AngII receptor action. Angiotensin II is present in high concentrations in follicular fluid (FF) and granulosa-lutein (GL) cells from patients at the time of egg retrieval (6–7). Angiotensin II receptors AT1 and AT2 have been identified by RIA and immunohistochemistry in human and other species’ ovarian follicular cells (8–10).

The OVRAS is implicated in a wide variety of phenomena taking place in the ovary, such as follicular growth and oocyte maturation, ovulation, steroidogenesis, angiogenesis, and follicular atresia (11–20). The OVRAS is linked to the plasminogen activator/plasminogen activator inhibitor system and both are regulated by gonadotropins (21). At midcycle, when FF plasminogen activator inhibitors are suppressed, apparently by the rise of FSH, the cleavage of renin substrate increases and the resultant AngII peak participates in the ovulation mechanism (13, 18–19). We showed that blockade of AngII receptors inhibits ovulation (11).

The role of the OVRAS in development of the follicular antrum and the formation of the cumulus oophorus remains unclear. However, gonadotropin-stimulated mural GL cells...
obtained from the FF of women at the time of egg retrieval for IVF undergo apoptosis (22–27), raising the possibility that the gonadotropin-driven OVRAS is involved in follicle cell dynamics and has a relationship with the developing oocyte. We demonstrated that GL cells undergo different degrees of apoptosis as a function of patients’ age (younger < older) and IVF outcome (unsuccessful > successful pregnancy) (28) and that AngII induces in vitro apoptosis of gonadotropin-exposed human IVF GL cells but not of cumulus cells, a mechanism which seems to be preferentially modulated by the AT2 receptor (29).

In addition to homeostatic regulation of cells and vessels, AngII receptors are implicated in a wide variety of AngII effects in the adult human ovary. These include steroidogenesis, ion fluxes, follicular growth and maturation, ovulation, angiogenesis and neovascularization and apoptosis (11–20). The active agents may be diverse members of the angiotensin peptide family, including angiotensin III and angiotensin-1–7, which may form in the absence of the angiotensin-converting enzyme (ACE1) and, therefore, is not affected by the clinically commonly used ACE inhibitors (30–31). The versatility of the AngII receptors and their presence in the follicular cells underline their possible importance in the function of the OVRAS. In this study we determined the relative abundance of AT1 and AT2 receptors in luteinized granulosa cells that had been harvested from previously diagnosed infertile women. Studying IVF patients with no ovarian factor (NOF) allowed comparison of AngII receptor expression with poor ovarian reserve (PR), endometriosis (ENDO) and polycystic ovary syndrome (PCOS) patients.

MATERIALS AND METHODS

We studied AngII receptor expression using real-time PCR for evaluation of mRNA produced per milligram of GL cells harvested at the time of follicular aspiration for IVF.

Reagents and Products

L-Glutamine, bovine serum albumin (BSA), amphotericin B, sodium bicarbonate, streptomycin sulphate, penicillin G, Percoll, modified McCoy’s 5A Medium, and Medium 199 with Hanks’ salts were purchased from Sigma-Aldrich Co. Ltd. (Madrid, Spain). Dynabeads M-450 CD45 was from Dynal Asa (Oslo, Norway). The SV Total RNA Isolation System was obtained from Promega Corporation (Madison, WI) and PowerScript Reverse Transcriptase was from Clontech (Mountain View, CA). Mini Opticon 3 Real-Time PCR System and SYBR Green Supermix were from Bio-Rad Laboratories (Hercules, CA).

Patients

Granulosa-lutein cells from 73 IVF cycles performed between January 2006 and April 2008 in a single private IVF center in La Laguna (Spain) were analyzed under a protocol approved by the Ethics Committee of the Universidad de La Laguna. For the purpose of this study, infertile patients were classified into the following diagnostic groups: control: NOF (tubal or male factor; n = 19); experimental: PR (day 3 plasma FSH >10 IU/mL) and older patients (> 40 years old with < 4 mature eggs, regardless of FSH; n = 19), ENDO (all stages, normal cycles, diagnosed by ultrasound and/or laparoscopy; n = 16) and PCOS (anovulation and ultrasound appearance, according to the Rotterdam criteria [32] n = 19). Demographics and cycle parameters for each group are specified in Table 1.

Induction of ovulation was carried out using recombinant FSH (Gonal F; Serono, Madrid, Spain), combined with recombinant LH (Luveris; Serono) or hMG (hMG-Lepori; Farma-Leopori, Madrid, Spain or Menopur; Ferring, Madrid, Spain). Doses were adjusted to the individual patient’s response, usually continuing until the two lead follicles had a mean diameter of 18 mm. The ovarian stimulation protocol was down-regulation in 15 patients, microflare in 20 patients of and antagonist in 38 patients. Ultrasound-guided egg retrieval was performed 36 hours after administration of 10.000 IU of hCG (hCG Lepori; Farma-Leopori, Madrid, Spain). The fertilization method for the retrieved oocytes was intracytoplasmic sperm injection (ICSI) in all cases. Embryo transfer was carried out with a Wallace catheter, under ultrasound guidance. One to three embryos were replaced in each cycle, depending on the patient’s age and embryo morphology. Embryo fragmentation was determined, in all cases, as the percentage of enucleated cell fragments < 45 μm seen in the embryo on day 3 of culture.

Isolation of GL Cells

Granulosa-lutein cells were collected from FF obtained during ultrasound-guided transvaginal oocyte retrieval and purified as previously described (28–29) immediately after egg retrieval. Briefly, all FF from each patient was pooled and the GL cells lightly centrifuged after removal of the oocyte. Cells were washed in “isolation medium” (Medium 199, supplemented with sodium bicarbonate [3.7 g/L], penicillin [59 mg/L], streptomycin [100 mg/L], amphotericin B [25 mg/L], L-glutamine [0.29 g/L] and BSA [0.1%]) and separated from red blood cells using a 50% Percoll gradient. Leukocytes were separated using anti-CD45-coated magnetic beads. The proportion of GL cells to erythrocytes always exceeded 95%. Cellular viability was obtained by trypan blue exclusion method in all cases exceeded 95%. The GL cells were re-washed, resuspended in modified McCoy’s 5A Medium (supplemented with L-glutamine [0.29 g/L] BSA [0.1%], penicillin [59 mg/L], streptomycin [100 mg/L], and amphotericin B [25 mg/L]), and frozen at −80°C until mRNA extraction.

RNA Extraction and Complementary DNA Synthesis

Total RNA from individual patients was extracted in batches of 10 by using the SV Total RNA Isolation System (Promega Corp., Madison, WI), following the manufacturer’s instructions. RNA obtained was reverse transcribed using PowerScript
### TABLE 1

Summary of patients' characteristics and IVF cycle parameters in the different subgroups studied.

<table>
<thead>
<tr>
<th></th>
<th>All (n = 73)</th>
<th>NOF (n = 19)</th>
<th>PR (n = 19)</th>
<th>ENDO (n = 16)</th>
<th>PCOS (n = 19)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (^a) (y)</td>
<td>34.33 ± 0.50</td>
<td>33.11 ± 0.70</td>
<td>35.79 ± 1.08</td>
<td>35.31 ± 1.22</td>
<td>33.26 ± 0.87</td>
<td>NS</td>
</tr>
<tr>
<td>Body mass index (^a)</td>
<td>23.74 ± 0.52</td>
<td>24.06 ± 0.98</td>
<td>23.23 ± 1.01</td>
<td>21.82 ± 0.72</td>
<td>25.54 ± 1.23</td>
<td>NS</td>
</tr>
<tr>
<td>Smokers (^b) (%)</td>
<td>23.3</td>
<td>26.3</td>
<td>15.8</td>
<td>31.3</td>
<td>26.3</td>
<td>NS</td>
</tr>
<tr>
<td>No. of IVF cycles/patient (^b)</td>
<td>1.42 ± 0.10</td>
<td>1.32 ± 0.13</td>
<td>1.37 ± 0.19</td>
<td>1.69 ± 0.28</td>
<td>1.37 ± 0.19</td>
<td>NS</td>
</tr>
<tr>
<td>Basal serum FSH level (^a) (mIU/mL)</td>
<td>8.59 ± 0.42</td>
<td>7.54 ± 0.63</td>
<td>11.27 ± 0.64</td>
<td>9.63 ± 1.15</td>
<td>6.12 ± 0.30</td>
<td>.000</td>
</tr>
<tr>
<td>Peak serum E2 level (^a) (pg/mL)</td>
<td>2,274.68 ± 135.41</td>
<td>2,633.89 ± 170.93</td>
<td>1,668.05 ± 204.80</td>
<td>2,387.13 ± 395.92</td>
<td>2,427.42 ± 266.75</td>
<td>NS</td>
</tr>
<tr>
<td>Duration of treatment (^a) (d)</td>
<td>10.58 ± 0.19</td>
<td>10.21 ± 0.39</td>
<td>10.68 ± 0.40</td>
<td>10.81 ± 0.33</td>
<td>10.63 ± 0.36</td>
<td>NS</td>
</tr>
<tr>
<td>Units of FSH administered (^a)</td>
<td>4,523.91 ± 268.19</td>
<td>4,104.47 ± 432.95</td>
<td>6,193.42 ± 403.74</td>
<td>5,732.81 ± 441.43</td>
<td>2,255.82 ± 315.31</td>
<td>.001</td>
</tr>
<tr>
<td>Units of LH administered (^a)</td>
<td>2,063.01 ± 175.25</td>
<td>1,849.34 ± 351.64</td>
<td>3,118.42 ± 276.45</td>
<td>2,662.50 ± 296.31</td>
<td>716.45 ± 168.43</td>
<td>.001</td>
</tr>
<tr>
<td>No. of oocytes retrieved (^a)</td>
<td>13.30 ± 1.05</td>
<td>16.05 ± 1.79</td>
<td>6.58 ± 0.75</td>
<td>10.00 ± 1.69</td>
<td>20.05 ± 2.29</td>
<td>.001</td>
</tr>
<tr>
<td>No. of MII oocytes (^a)</td>
<td>9.99 ± 0.86</td>
<td>11.00 ± 1.34</td>
<td>4.37 ± 0.64</td>
<td>8.44 ± 1.38</td>
<td>15.89 ± 1.99</td>
<td>.001</td>
</tr>
<tr>
<td>Embryo fragmentation (^b) (%)</td>
<td>9.74 ± 1.10</td>
<td>8.95 ± 1.43</td>
<td>9.38 ± 3.07</td>
<td>9.76 ± 2.57</td>
<td>10.83 ± 1.74</td>
<td>NS</td>
</tr>
<tr>
<td>Pregnancy rate (^b) (%)</td>
<td>46.6%</td>
<td>57.9%</td>
<td>36.8%</td>
<td>18.8%</td>
<td>68.4%</td>
<td>.017</td>
</tr>
</tbody>
</table>

**Note:** All data are expressed either as mean ± SE or as percentage. Comparisons were made between the four groups of patients (NOF, PR, ENDO) considered. MII = metaphase II; NS = not statistically significant; NOF = no ovarian factor; ENDO = endometriosis; PCOS = polycystic ovary syndrome; PR = poor ovarian reserve.

\(^a\) Analysis of variance: NS; \(P > .05\).

\(^b\) Kruskal-Wallis test: NS; \(P > .05\).

Reverse Transcriptase (Clontech, Mountain View, CA), which eliminates RNase H activity, allowing synthesis of a higher percentage of full-length cDNA. Total of RNA was reverse transcribed in 90 μL as follows: 0.5 μg of Oligo dT20VN was incubated at 65°C for 2 minutes. The subsequent reverse transcription was performed at 42°C for 90 minutes by adding 5× First-Strand Buffer, 100 mmol/L DTT, 10 mM dNTPs, 40 IU RNasin inhibitor (Promega Corp.), and 100 IU of PowerScript Reverse Transcriptase (Clontech). The reverse transcription was inactivated by heating at 72°C for 15 minutes. The cDNA samples were frozen and kept at −80°C until further analysis.

Quantitative Real-Time Reverse-Transcription PCR
Quantitative fluorescent real-time PCR reactions were carried out in a Mini Opticon 3 Real-Time PCR System (Bio-Rad Laboratories, Hercules, CA) using the SYBR Green Supermix (Bio-Rad Laboratories). The specific primers used for each gene were: β-actin (F = CTTCTTCTCGGCGATGG; R = GCCGCCAGACAGCACTGT), AT1 (F = GCCGAAGAGACATTCCCTCTGC; R = CAGCGCTCATCTGCTTAATGC), and AT2 (F = AGAACAGGATAACCCGTGACC; R = AGGATGGCAGAAGGATGCG). The amplification reactions were performed in a final volume of 22 μL, containing 2× Sybr Green Supermix (100 mmol/L KCl, 40 mmol/L Tris-HCl pH 8.4, 0.4 mmol/L of each dNTP, 50 U/mL iTaq DNA polymerase, 6 mmol/L MgCl2, SYBR Green I, 20 nmol/L of PowerScript Reverse Transcriptase (Clontech). The reverse transcription was inactivated by heating at 72°C for 15 minutes. The cDNA samples were frozen and kept at −80°C until further analysis.

After initial incubation at 95°C for 10 minutes to activate the Taq DNA polymerase, templates of all the specific transcripts were amplified for 45 cycles at 95°C for 20 seconds, 59°C for 20 seconds for all primers and 72°C for 30 seconds. A final amplification step for 1 minute was added at 72°C at the end of the PCR. Single product formation was verified using the melting point and agarose gel electrophoresis after completion of PCR. The melting point program was carried out from 65°C to 95°C with a heating rate of 0.1°C/s and reading every 1°C. Each sample was analyzed in triplicate, and water blanks were included in each experiment. The housekeeping gene β-actin was amplified as a reference in all of the experiments for mRNA quantification. Because cellular actin expression is relatively constant, the AT1 and AT2 receptors result was converted to the ratio of AngII receptor/β-actin PCR product for each measurement.

Statistical Analysis
Results are expressed as mean ± standard error. Statistical analysis was performed with SPSS software, using Pearson’s correlation coefficients, one-way analysis of variance, Kruskal-Wallis test, Student’s t test and Mann-Whitney U test. A P value < .05 was considered to be statistically significant. Analysis of the data by Kolmogorov-Smirnov test showed that the expression of AT1 and AT2 receptors did not follow a normal Gaussian distribution; therefore, to use Pearson’s correlation coefficients, the data were transformed to decimal logarithm values.

RESULTS
Grand analysis of the individual measurements from all cycles showed a higher expression of AT1 receptor mRNA compared with AT2 receptor mRNA (7.44 ± 0.77 vs. 1.09 ± 0.34) and a positive linear correlation between the two AngII receptors’ expression (r = 0.31; P < .01; Fig. 1A). Furthermore, the AT1/AT2 ratio was negatively correlated with the patients’ age (r = −0.25; P < .05; Fig. 1B). The AT1 and AT2 receptor expression levels did not show any correlation with body mass index, smoking status, number of previous IVF attempts, gravidity, parity, ovulation induction protocol, or the pregnancy rate during the cycle studied.

Analysis of subgroups did not detect statistically significant differences for AT1 or AT2 receptor expression in patients with different infertility diagnosis (Table 2). There were trends toward higher expression of AT1 and AT2 receptor mRNA in NOF and PCOS women. Relevant correlations found in the different diagnostic categories are depicted in Table 3.

No Ovarian Factor
In patients with NOF we found a strong positive correlation between AT1 and AT2 receptor mRNA expression (r = 0.46; P < .05; Fig. 1C), which was not detected in patients with other infertility factors. A negative correlation between AT1 receptor mRNA expression and both embryo fragmentation (r = −0.57; P < .05) and the number of mature oocytes retrieved (r = −0.63; P < .01) was also detected in the NOF group (Figs. 1D and 1E).

Poor Ovarian Reserve
In PR patients AT2 receptor mRNA expression correlated negatively with the number of MII oocytes retrieved (r = −0.55; P < .05; Fig. 1F) and correlated positively with both the number of days of ovarian stimulation (r = 0.52; P < .05) and the amount of FSH and LH administered for ovulation induction (FSH: r = 0.63; P < .01; LH: r = 0.62; P < .01; Fig. 1G). In the same patients, AT1 receptor mRNA expression correlated positively with day 3 FSH level (r = 0.49; P < .05). The AT1/AT2 ratio correlated positively with the number of total oocytes recovered (r = 0.61; P < .01) and with the number of MII oocytes (r = 0.68; P < .01).

Polycystic Ovarian Syndrome
In the PCOS subgroup there were negative correlations between AT1 receptor expression and the amount of FSH administered for induction of ovulation (r = −0.49; P < .05) and patients’ age (r = −0.56; P < .05; Fig. 1H). Negative correlations between AT1/AT2 ratio and patients’ age (r = −0.46; P < .05) and day 3 FSH level (r = −0.49; P < .05) could also be detected.
Correlations observed between angiotensin II type 1 (AT1) and type 2 (AT2) receptor mRNA expression and IVF variables in all patients and women with no ovarian factor (NOF), poor ovarian reserve (PR) and polycystic ovary syndrome (PCOS). MII = metaphase II.

Endometriosis
In ENDO patients there were no significant relations between AngII receptor expression and the clinical or demographic parameters.

DISCUSSION
This study shows for the first time in humans that the average relative expression of AT1 receptor mRNA in pooled mural GL cells from individual IVF patients is higher than that of AT2 receptor mRNA. This is true for both women with normal ovarian function and women with ovarian pathology. Furthermore, there is a linear positive correlation between AT1 and AT2 receptors expression in the whole group of patients and in NOF, which is not found in women with ovarian pathology. Women with no ovarian factor had a strong linear correlation between AT1 and AT2 receptor expression. The weaker correlation in the other conditions (PR, ENDO, and PCOS) could reflect underlying genetic or epigenetic conditions, altering the ovarian milieu, which modify the physiologic expression of AngII receptors and/or AngII formation and action. Thus, the complex role of AngII in ovarian folliculogenesis is disrupted in these disease states. We have to keep in mind that besides AT1 and AT2 receptors expression, physiologic effects of OVRAS depend on a number of situations that may be modified in different disease states, such as differential binding/affinity of the receptors, signal transduction pathways and/or effect of the different members of the angiotensin family, such as angiotensin-(1–7) and angiotensin III, on their specific receptors. This may account for some apparent contradictions in the observed AngII effects in some situations. In the whole group of patients, the AT1/AT2 ratio decreases with increasing patient’s age, indicating that aging is associated with increased relative expression of the AT2 receptor.

The OVRAS has been shown to play key roles in ovarian physiology. The preovulatory increase in intrafollicular AngII may facilitate oocyte maturation and ovulation or the production of progesterone by human GL cells. Angiotensin

### TABLE 2

<table>
<thead>
<tr>
<th></th>
<th>All (n = 73)</th>
<th>NOF (n = 19)</th>
<th>PR (n = 19)</th>
<th>ENDO (n = 16)</th>
<th>PCOS (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1 expression</td>
<td>7.44 ± 0.77</td>
<td>8.65 ± 1.84</td>
<td>5.72 ± 1.06</td>
<td>6.53 ± 1.44</td>
<td>8.76 ± 1.64</td>
</tr>
<tr>
<td>AT2 expression</td>
<td>1.09 ± 0.34</td>
<td>1.16 ± 0.56</td>
<td>0.54 ± 0.21</td>
<td>0.60 ± 0.30</td>
<td>1.96 ± 1.15</td>
</tr>
</tbody>
</table>

*Note: Abbreviations as in Table 1.*


### TABLE 3

Summary of the significant correlations observed between AT1 and AT2 receptor mRNA expression and IVF variables in the different subgroups of patients.

<table>
<thead>
<tr>
<th></th>
<th>NOF (n = 19)</th>
<th>PR (n = 19)</th>
<th>PCOS (n = 19)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AT1 vs. AT2</td>
<td>r = 0.46; P&lt;.05</td>
<td></td>
<td></td>
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<tr>
<td>AT1 vs. embryo fragmentation</td>
<td>r = −0.57; P&lt;.05</td>
<td></td>
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</tr>
<tr>
<td>AT1 vs. MII oocytes</td>
<td>r = −0.63; P&lt;.01</td>
<td></td>
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</tr>
<tr>
<td>AT1 vs. day +3 FSH</td>
<td></td>
<td>r = 0.49; P&lt;.05</td>
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<tr>
<td>AT2 vs. days of treatment</td>
<td></td>
<td>r = 0.52; P&lt;.05</td>
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<tr>
<td>AT2 vs. MII oocytes</td>
<td></td>
<td>r = −0.55; P&lt;.05</td>
<td></td>
</tr>
<tr>
<td>AT2 vs. FSH used</td>
<td></td>
<td>r = 0.63; P&lt;.01</td>
<td></td>
</tr>
<tr>
<td>AT2 vs. LH used</td>
<td></td>
<td>r = 0.62; P&lt;.01</td>
<td></td>
</tr>
<tr>
<td>AT1/AT2 ratio vs. no. of oocytes</td>
<td>r = 0.61; P&lt;.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AT1/AT2 ratio vs. MII oocytes</td>
<td></td>
<td>r = 0.68; P&lt;.01</td>
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<tr>
<td>AT1 vs. age</td>
<td></td>
<td></td>
<td>r = −0.56; P&lt;.05</td>
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<tr>
<td>AT1 vs. FSH used</td>
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<tr>
<td>AT1/AT2 ratio vs. age</td>
<td></td>
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<td>r = −0.46; P&lt;.05</td>
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<tr>
<td>AT1/AT2 ratio vs. day 3 FSH</td>
<td></td>
<td></td>
<td>r = −0.49; P&lt;.05</td>
</tr>
</tbody>
</table>

*Note: Abbreviations as in Table 1.*

II is increased in both follicular atresia and corpus luteum regression, events that are known to be linked to apoptosis, an action very prominent in PCOS, in which high levels of AngII are expressed in the cystic follicles mural granulosa cells (33–35). Using a rat model of PCOS we observed that apoptosis is involved in the formation of cystic follicles (35). An increased level of AT2 receptor expression in the PCOS subgroup of patients would be in accordance with a role for AngII in the process of accelerated follicular atresia/apoptosis and cyst formation typical of this syndrome.

The existence of a negative correlation between AT1 receptor mRNA expression and embryo fragmentation in patients with normal ovaries (NOF) deserves attention. We have previously shown that AngII causes GL cell apoptosis, preferentially via the AT2 receptor (29), and that increased GL cell apoptosis is associated with increased embryo fragmentation (28). The AT1 receptor promotes generalized positive effects in growth and development, in general, anti-apoptotic effects. The present finding of decreased AT1 receptor expression in patients with higher embryo fragmentation is consistent with a role of AngII in GL cell apoptosis. Within each group, the extent of embryo fragmentation did not differ (Table 1).

The correlations found between AngII receptor expression and the number of mature oocytes retrieved seems to reflect the importance of a correct AT1/AT2 ratio for normal follicular function. In NOF, increased expression of AT1 receptor is associated with lower number of MII oocytes. In PR, increased expression of AT2 receptor is associated with lower number of MII oocytes.

In summary, the existence of a proper balance between proliferation and apoptosis in GL cells is a prerequisite for obtaining a fully competent oocyte. The data presented in this paper suggest that a correct balance between AT1 and AT2 receptors expression may participate in the regulation of proliferation and apoptosis in the human follicle. The altered expression of AT1 and AT2 receptors observed in PCOS and PR patients might be cause or effect of the sub-optimal follicular development seen in these conditions. Further studies are needed to better clarify these associations and cause-effect relationships.

REFERENCES


