Glutathione S-transferases in human ovarian follicular fluid

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Objective: To study the levels of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 in human preovulatory ovarian follicular fluid (FF) and pooled granulosa and cumulus cells.

Design: The relation of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 with P and 17β-E2 concentrations were studied.

Setting: The Department of Obstetrics and Gynecology, the Department of Gastroenterology, and the Laboratory of Endocrinology and Reproduction of the University Hospital Nijmegen in Nijmegen, the Netherlands.

Patient(s): Infertile women participating in an IVF program.

Result(s): Detectable amounts of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 were found in ovarian FF and pooled cumulus and granulosa cells. Concentrations of glutathione S-transferase Alpha 1-1 were always much higher than those of glutathione S-transferase Pi 1-1. Both ovarian FF concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 did not correlate with ovarian FF concentrations of 17β-E2 and P.

Conclusion(s): The high FF concentrations of glutathione S-transferase Pi 1-1 and especially of glutathione S-transferase Alpha 1-1 suggest that these enzymes may play an important role in the detoxification processes in the follicles. The lack of correlation between follicular P and 17β-E2 and glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 indicates that both enzymes presumably are not present as a result of the high steroid levels. (Fertil Steril® 1997;68:907–11. © 1997 by American Society for Reproductive Medicine.)

Key Words: Ovarian follicular fluid, glutathione S-transferase, steroids, in vitro fertilization program

Follicular fluid (FF) is formed by a gradual influx of fluid derived from the blood and by compounds synthesized and secreted by theca and granulosa cells. Therefore, its composition reflects both the plasma composition and the secretory activity of theca and granulosa cells (1). Follicular fluid in humans and animals contains much higher concentrations of both P and 17β-E2 than does blood (2).

The glutathione S-transferases (EC 2.5.1.18) are a multigene family of enzymes that catalyze the nucleophilic addition of glutathione to the electrophilic centers of a wide variety of xenobiotics. They also serve as transport proteins for a broad range of lipophilic compounds, such as bilirubin, bile acids, and steroid hormones. Glutathione S-transferase Alpha seems to be associated closely with the glutathione-dependent Δ5-3-ketosteroid isomerase, which catalyzes the conversion of pregnenolone to P and of dehydroepiandrosterone to androstenedione, together with 3β-hydroxysteroid dehydrogenase (3). On the basis of their structural, physicochemical, enzymatic, and immunologic properties, the family of en-
zymes is divided into four classes: Alpha, Mu, Pi, and Theta, each of which consists of one or more isoforms (4, 5).

Glutathione S-transferases have been shown to be present in ovarian luteal tissue in pig (6) and rat ovaries (3) as well as in normal human ovarian tissue (7, 8). Immunohistochemical staining for glutathione S-transferase Alpha and glutathione S-transferase Pi in human ovaries revealed that granulosa cells in granulafollicles and corpora lutea, but not in primordial, primary, or atretic follicles, stained for glutathione S-transferase Alpha and, to a lesser extent, for glutathione S-transferase Pi. Glutathione S-transferase Alpha strikingly parallels sites of steroid production.

To our knowledge, the presence of glutathione S-transferases in human FF never has been reported. Therefore, we initiated this study to determine the presence and concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1, isoforms of the class Alpha and class Pi glutathione S-transferases, respectively, in human preovulatory FF.

Further, we determined the correlation of both glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 with ovarian FF concentrations of 17β-E2 and P to investigate the putative role of these enzymes in the detoxification or transport of the very high steroid levels. We also determined glutathione S-transferase concentrations in pooled granulosa and cumulus cells to find a possible origin for glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 in the FF.

MATERIALS AND METHODS

Patients

After informed consent was given, ovarian FF samples were obtained from 10 women participating in an IVF program. The experimental protocol was approved by the Medical Ethical Review Committee of the University Hospital Nijmegen.

Multiple follicular development was induced by the administration of hMG (Humigon; Organon, Oss, the Netherlands) as described previously (9). A GnRH agonist (1 mg, Lucrin; Abbot, Amstelveen, the Netherlands) was administered SC from day 21 of the preceding cycle until the evening of hCG (Pregnyl; Organon) injection. The dosage of hMG was based on the serum E2 concentration and varied between 150 and 300 IU/d.

As soon as the serum E2 concentration reached approximately 800 pmol/L per follicle and the diameter of the follicle reached >15 mm, 10,000 IU of hCG was injected IM. Follicular fluid was aspirated by transvaginal puncture using ultrasound guidance and fluid from three follicles was obtained from each patient. The FF was centrifuged separately for 10 minutes at 3,000 × g to remove red blood cells. The supernatant was frozen and stored at −20°C until analysis. The FF was examined separately for glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 and for their corresponding E2 and P concentrations. From each patient, the follicle with the highest E2 concentration was used for statistical evaluation.

Forty oocyte–corona–cumulus cell complexes were isolated from follicular aspirates. They were visualized under Leitz (Dialux EB 20, Wetzlar, Germany) optics to establish the morphology. Only mature cumulus cell complexes were used (10). Cumulus cells from all complexes were placed separately in 2 mL of human tubal fluid (HTF) medium and were washed three times in the same volume of HTF (9). Thereafter, the cumulus cells were pooled, placed in 10 mL of HTF, and centrifuged at 300 × g for 10 minutes.

The granulosa cells were isolated from clear FF aspirates that were visually free of blood. Naturally, the aspirated cells contained red blood cells. These were removed almost completely by allowing them to settle in 100-mm Petri dishes (Falcon 1001) containing 5 mL of HTF for 10 minutes, whereafter the granulosa cells were transferred using a Pasteur pipette to a new Petri dish with fresh HTF.

This procedure was performed three times and then the cells were pooled, placed in 10 mL of HTF, and centrifuged at 300 × g for 10 minutes. The cells were homogenized in 10 vol of buffer (250 mM of sucrose, 20 mM of Tris-HCl, and 1 mM of dithiothreitol; pH 7.4) with 10 strokes in small glass-glass tissue grinders on ice. The homogenates were centrifuged at 150,000 × g at 4°C for 1 hour. The supernatants were frozen at −70°C until analysis.

Blood plasma concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 also were determined in the blood plasma samples of 20 patients taken during follicle puncture. Antecubital venous blood samples were taken in the sitting position after 5 minutes of rest in 4-mL ethylenediaminetetraacetic acid tubes (no 606601; Becton Dickinson, Grenoble, France). The blood was centrifuged at 3,000 × g for 10 minutes at 20°C. The plasma was removed and stored at −20°C until analysis.

Assays

Glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 levels were determined
using recently developed ELISAs (11, 12). Briefly, microtiter plates were coated overnight with purified monoclonal antibody. One hundred microliters of standard (0.08–20 μg/L for glutathione S-transferase Alpha 1-1; 0.4–100 μg/L for glutathione S-transferase Pi 1-1) or 1:1 diluted plasma samples or 1:1 and 1:100 diluted FF samples were added to the wells and incubated overnight. The next day, the plates were washed and incubated with rabbit anti-glutathione S-transferase Alpha 1-1 or anti-glutathione S-transferase Pi 1-1 antiserum. They were washed again and were incubated with horseradish peroxidase-labeled swine anti-rabbit antigen. After a final wash, the plates were stained with o-phenylenediamine–H2O2.

The glutathione S-transferase Alpha 1-1 assay has a minimum detectable concentration of <0.04 μg/L and intra-assay and interassay coefficients of variation of 2.5% and 7.3%, respectively. In the case of glutathione S-transferase Pi 1-1, these values are 0.4 μg/L, 5.8%, and 10.9%, respectively. Dilutions of these FF samples were parallel to the standard curves and the addition of 3,700 nmol/L of E2 or 1,600 nmol/L of P had no effect on the standard curves of the glutathione S-transferase Alpha 1-1 or glutathione S-transferase Pi 1-1 assays.

Determination of P and E2 concentrations were performed after diethyl ether extraction and Sephadex LH-20 chromatography (Pharmacia, Woerden, the Netherlands) in slightly modified RIAs as described previously (13). The minimum detectable concentrations of the assays were 10 pmol/L for E2 and 1.3 nmol/L for P. Intra-assay and interassay coefficients of variation were 5.4% and 8.4%, respectively. Concentrations of the assays were determined significantly with glutathione S-transferase Alpha 1-1 concentrations were correlated significantly with follicular P and E2 (r = 0.10, P > 0.1, respectively) or E2 (r = 0.15, P > 0.1, respectively) concentrations. Follicular glutathione S-transferase Alpha 1-1 concentrations were correlated significantly with glutathione S-transferase Pi 1-1 concentrations (r = 0.93, P = 0.0001). The median concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 in the blood plasma of 20 patients sampled concurrently at the moment of follicle puncture were 1.71 μg/L (range, 0.95–3.57 μg/L) and 6.30 μg/L (range, 5.4–104 μg/L), respectively. These plasma concentrations fell within the normal reference ranges for healthy women of comparable age (11, 12).

**DISCUSSION**

This study shows that glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 are present in considerable amounts in human ovarian FF. Glutathione S-transferase Alpha 1-1 is present in human ovarian FF, and the concentrations were correlated significantly with follicular P and E2. Glutathione S-transferase Pi 1-1 concentrations were correlated significantly with follicular P and E2.

### Table 1 GSTA1-1, GSTP1-1, P, and 17β-E2 in Human Preovulatory FF

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>GSTA1-1 (μg/L)</th>
<th>GSTP1-1 (μg/L)</th>
<th>P (nmol/L)</th>
<th>17β-E2 (nmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>252</td>
<td>17</td>
<td>44</td>
<td>5.8</td>
</tr>
<tr>
<td>2</td>
<td>582</td>
<td>29</td>
<td>56</td>
<td>3.9</td>
</tr>
<tr>
<td>3</td>
<td>468</td>
<td>17</td>
<td>41</td>
<td>4.4</td>
</tr>
<tr>
<td>4</td>
<td>485</td>
<td>21</td>
<td>56</td>
<td>1.0</td>
</tr>
<tr>
<td>5</td>
<td>495</td>
<td>17</td>
<td>54</td>
<td>3.3</td>
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<tr>
<td>6</td>
<td>212</td>
<td>15</td>
<td>46</td>
<td>2.6</td>
</tr>
<tr>
<td>7</td>
<td>3509</td>
<td>140</td>
<td>18</td>
<td>2.7</td>
</tr>
<tr>
<td>8</td>
<td>253</td>
<td>17</td>
<td>27</td>
<td>1.4</td>
</tr>
<tr>
<td>9</td>
<td>182</td>
<td>13</td>
<td>33</td>
<td>1.8</td>
</tr>
<tr>
<td>10</td>
<td>774</td>
<td>24</td>
<td>44</td>
<td>2.6</td>
</tr>
<tr>
<td>Median</td>
<td>477</td>
<td>17</td>
<td>44</td>
<td>2.7</td>
</tr>
<tr>
<td>Range</td>
<td>182–3,609</td>
<td>13–140</td>
<td>13–56</td>
<td>1.0–5.8</td>
</tr>
</tbody>
</table>

### Table 2 GSTA1-1 and GSTP1-1 in Pooled Cumulus and Granulosa Cells

<table>
<thead>
<tr>
<th>Tissue</th>
<th>GSTA1-1 (μg/mg protein)</th>
<th>GSTP1-1 (μg/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulus</td>
<td>1.42</td>
<td>0.082</td>
</tr>
<tr>
<td>Granulosa</td>
<td>12.84</td>
<td>0.35</td>
</tr>
</tbody>
</table>

**Correlations between FF E2, P, and glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 were computed using Spearman’s rank correlation test.**

**RESULTS**

Follicular glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 were detectable in all patients studied. Their concentrations as well as those of P and E2 are presented in Table 1. The concentrations of glutathione S-transferase Alpha 1-1 were approximately 30 times higher than those of glutathione S-transferase Pi 1-1. Glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 also were detected in pooled cumulus and granulosa cells (Table 2). Again, the concentrations of glutathione S-transferase Alpha 1-1 were much higher than those of glutathione S-transferase Pi 1-1 (17 and 36 times, respectively). In addition, the enzyme concentrations of granulosa cells were much higher than those of cumulus cells.

Neither follicular glutathione S-transferase Alpha 1-1 nor glutathione S-transferase Pi 1-1 concentrations were correlated significantly with follicular P (r = 0.10, P > 0.1, and r = 0.14, P > 0.1, respectively) or E2 (r = 0.15, P > 0.1, and r = 0.16, P > 0.1, respectively) concentrations. Follicular glutathione S-transferase Alpha 1-1 concentrations were correlated significantly with glutathione S-transferase Pi 1-1 concentrations (r = 0.93, P = 0.0001). The median concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 in the blood plasma of 20 patients sampled concurrently at the moment of follicle puncture were 1.71 μg/L (range, 0.95–3.57 μg/L) and 6.30 μg/L (range, 5.4–104 μg/L), respectively. These plasma concentrations fell within the normal reference ranges for healthy women of comparable age (11, 12).
in far higher concentrations than glutathione S-transferase Pi 1-1 in both FF and pooled cumulus and granulosa cells. Median concentrations of both enzymes are higher in the follicles of patients undergoing IVF than in their blood plasma. The level of glutathione S-transferase Alpha 1-1 especially is far above the blood plasma level, indicating a less important role for the influx of this enzyme from blood. Remarkably, the concentration of glutathione S-transferase Alpha 1-1 in FF compared with that in blood plasma (FF/plasma ratio: ±280) is much more pronounced than that of glutathione S-transferase Pi 1-1 (ratio: ±2.7). This finding is in concordance with the much higher concentrations of glutathione S-transferase Alpha 1-1 in both granulosa and cumulus cells compared with the concentrations of glutathione S-transferase Pi 1-1.

Using immunohistochemical techniques, Rahilly et al. (8) have demonstrated that glutathione S-transferase Alpha is localized to the steroid-producing cells. We found that glutathione S-transferase concentrations are higher in granulosa cells than in cumulus cells (Table 2). Therefore, it is conceivable that granulosa cells are the main source of the follicular enzyme concentrations.

The functions of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 in ovarian physiology remain unclear. Glutathione S-transferase Alpha is associated closely with the glutathione-dependent Δ5-3-ketosteroid isomerase that catalyzes the conversion of pregnenolone to P and of dehydroepiandrosterone to androstenedione, together with 3β-hydroxysteroid dehydrogenase (3). Glutathione S-transferase Alpha expression was found immunohistochemically in human granulosa cells during the second half of the preovulatory phase and in corpus luteum tissue, correlating well with sites of Δ5-3-ketosteroid isomerase activity (8).

Glutathione S-transferase Alpha also was found in cultured pig granulosa cells. In these cells, glutathione S-transferase Alpha concentrations increased after stimulation by gonadotropins, in association with the production of P (6). However, two studies indicated the independence of glutathione S-transferase activity from hormonal variations, whereas cytochrome P-450-dependent hydroxylases seemed to fluctuate with hormonal variations (15, 16).

The lack of any correlation between follicular glutathione S-transferases and steroid hormones in the same preovulatory phase in women participating in an IVF program, as found in our study, could point to a role for glutathione S-transferases as detoxifying enzymes that protect the ovum from reactive oxygen species and xenobiotics. Reactive oxygen species may be released in connection with follicle rupture, because inhibition of oxygen free radicals leads to inhibition of ovulation (17). Reactive oxygen species are thought to play a role in oocyte maturation (18). Polychlorinated biphenyls (19, 20) and hexachlorobenzene (21), both notorious xenobiotics, have been demonstrated in the FF of patients undergoing IVF and are known to induce glutathione S-transferases in tissues other than the ovaries (22, 23).

In conclusion, high concentrations of glutathione S-transferase Alpha 1-1 and glutathione S-transferase Pi 1-1 were found in the FF as well as the cumulus and especially the granulosa cells of women undergoing IVF, suggesting that these enzymes may play a role in detoxification processes in the follicular microenvironment.

REFERENCES