Relationship between steroid concentrations in ovarian follicular fluid and oocyte morphology in patients undergoing intracytoplasmic sperm injection (ICSI) treatment

P. Xia¹ and E. V. Younglai²

¹Department of Obstetrics and Gynecology, University of Arizona, Tucson, Arizona 85724, USA; and ²Department of Obstetrics and Gynecology, McMaster University, Hamilton, Ontario L8N 3Z5, Canada

The objective of this study was to investigate the relationship between oocyte morphology and follicular fluid steroid concentrations in patients being treated with intracytoplasmic sperm injection. A total of 82 IVF cycles were evaluated in patients aged 24–40 years. Oocytes at metaphase II were graded into four groups according to the status of the first polar body and the size of the perivitelline space. The proportion of oocytes at the germinal vesicle and germinal vesicle breakdown stages, and the proportion of degenerated oocytes and oocytes with a large polar body were compared with different concentrations of oestradiol, progesterone and testosterone in the follicular fluid. The association between these oocyte characteristics and the ratio of oestradiol:testosterone and oestradiol:progesterone was also analysed. The results showed that oocyte morphology, as assessed by the status of the first polar body and the size of the perivitelline space, is associated with the ratio of oestradiol:testosterone and oestradiol:progesterone but not with the absolute concentrations of oestradiol, progesterone and testosterone in the follicular fluid. A ratio of oestradiol:testosterone > 200 is the best indicator for a small proportion of grade 1 and 2 oocytes (poor quality), a large proportion of grade 3 and 4 oocytes (good quality), and a small proportion of oocytes with cytoplasmic inclusions. These results will be of clinical use in evaluating oocyte quality.

Materials and Methods

This study was approved by the Committees of Chedoke-McMaster Hospitals Foundation and also the F. L. Johnson

Received 1 September 1999.

© 2000 Journals of Reproduction and Fertility Ltd
0022–4251/2000
Downloaded from Bioscientifica.com at 10/23/2018 06:47:10AM via free access
Foundation at the McMaster University and the Institutional Ethics Review Board. A total of 82 patients, who underwent ICSI treatment in the in vitro fertilization (IVF) programme at the Department of Obstetrics and Gynecology, Health Sciences Center, McMaster University, were included in this study after informed consent of the patients was obtained. Female age ranged between 24 and 40 years. The patients were classified into three ranges: 0–999, 1000–1999 and 2000–3205 ng ml–1 for oestradiol; 0–20, 21–30 and 31–117 ng ml–1 for progesterone; and 0–5, 5.1–10 and 11–19 ng ml–1 for testosterone in the follicular fluid.

Statistical analysis

Statistical comparisons were performed by one-way ANOVA with the use of Statistix software (Tallahassee, FL). P values < 0.05 were considered significant.

Results

Oocyte morphology and absolute concentrations of oestradiol, progesterone and testosterone in follicular fluid

The proportion of oocytes at grades 1 and 2 (poor morphology) and grade 3 and 4 (good morphology) was not correlated with the absolute concentrations of oestradiol.
progesterone and testosterone in follicular fluid (Tables 1, 2 and 3). The proportion of oocytes with a large polar body was lower at oestradiol concentrations in the range 1000–1999 ng ml⁻¹ and testosterone in the range 5–10 ng ml⁻¹ (P < 0.05) (Tables 1 and 3). There was a higher percentage of oocytes at the germinal vesicle and GVBD stages (18%) when progesterone was at a low concentration (0–20 ng ml⁻¹) compared with the percentage (11%) at 31–117 μg progesterone ml⁻¹ (P < 0.05, Table 2).

Table 3. Oocyte morphology and testosterone concentrations in human follicular fluid

<table>
<thead>
<tr>
<th>Testosterone (ng ml⁻¹)</th>
<th>Group size</th>
<th>Percentage of grade 1 and 2 oocytes</th>
<th>Percentage of grade 3 and 4 oocytes</th>
<th>Percentage of GV and GVBD oocytes</th>
<th>Percentage of degenerated oocytes</th>
<th>Percentage of oocytes with large polar body</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>21</td>
<td>32.20 ± 18.39</td>
<td>31.75 ± 18.25</td>
<td>14.46 ± 9.80</td>
<td>13.49 ± 10.58</td>
<td>7.83 ± 9.88a</td>
</tr>
<tr>
<td>5.1–10</td>
<td>48</td>
<td>35.66 ± 26.12</td>
<td>32.41 ± 25.15</td>
<td>12.68 ± 12.02</td>
<td>15.10 ± 15.02</td>
<td>2.76 ± 6.03b</td>
</tr>
<tr>
<td>11–19</td>
<td>13</td>
<td>35.67 ± 21.11</td>
<td>27.96 ± 18.17</td>
<td>12.64 ± 7.98</td>
<td>17.46 ± 13.43</td>
<td>9.65 ± 10.72a</td>
</tr>
<tr>
<td>Total</td>
<td>82</td>
<td>34.78 ± 23.65</td>
<td>31.54 ± 22.60</td>
<td>13.13 ± 10.96</td>
<td>15.06 ± 13.78</td>
<td>5.15 ± 7.99</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

abValues (two patient values were missing) in the same column with different superscripts are significantly different (P < 0.05).

GV: oocytes in the germinal vesicle stage; GVBD: oocytes in germinal vesicle breakdown.

Table 4. Oocyte morphology and oestradiol:testosterone ratios in human follicular fluid

<table>
<thead>
<tr>
<th>Oestradiol: testosterone</th>
<th>Group size</th>
<th>Percentage of grade 1 and 2 oocytes</th>
<th>Percentage of grade 3 and 4 oocytes</th>
<th>Percentage of GV and GVBD oocytes</th>
<th>Percentage of degenerated oocytes</th>
<th>Percentage of oocytes with large polar body</th>
<th>Percentage of oocytes with cytoplasmic inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–100</td>
<td>25</td>
<td>43.52 ± 22.40</td>
<td>26.72 ± 24.78</td>
<td>9.12 ± 10.17</td>
<td>15.11 ± 14.76</td>
<td>4.72 ± 6.44</td>
<td>21.66 ± 22.09a</td>
</tr>
<tr>
<td>151–200</td>
<td>13</td>
<td>27.19 ± 17.03</td>
<td>34.93 ± 20.58</td>
<td>11.42 ± 7.42</td>
<td>17.10 ± 15.86</td>
<td>8.40 ± 11.88</td>
<td>13.20 ± 12.62a</td>
</tr>
<tr>
<td>&gt;200</td>
<td>14</td>
<td>20.86 ± 16.17</td>
<td>42.21 ± 24.65</td>
<td>16.66 ± 12.07</td>
<td>13.01 ± 7.89</td>
<td>5.33 ± 10.78</td>
<td>9.61 ± 11.57a</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>35.02 ± 22.51</td>
<td>30.99 ± 21.96</td>
<td>13.23 ± 10.57</td>
<td>15.25 ± 13.95</td>
<td>5.17 ± 8.51</td>
<td>14.90 ± 17.85</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

abValues (two patient values were missing) in the same column with different superscripts are significantly different (P < 0.05).

GV: oocytes in the germinal vesicle stage; GVBD: oocytes in germinal vesicle breakdown.

Table 5. Oocyte morphology and oestradiol:progesterone ratios in human follicular fluid

<table>
<thead>
<tr>
<th>Oestradiol: progesterone</th>
<th>Group size</th>
<th>Percentage of grade 1 and 2 oocytes</th>
<th>Percentage of grade 3 and 4 oocytes</th>
<th>Percentage of GV and GVBD oocytes</th>
<th>Percentage of degenerated oocytes</th>
<th>Percentage of oocytes with large polar body</th>
<th>Percentage of oocytes with cytoplasmic inclusions</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–20</td>
<td>21</td>
<td>39.45 ± 25.95</td>
<td>30.44 ± 26.53</td>
<td>10.18 ± 10.37</td>
<td>13.87 ± 14.24</td>
<td>5.39 ± 7.65</td>
<td>25.74 ± 23.73a</td>
</tr>
<tr>
<td>21–40</td>
<td>32</td>
<td>35.69 ± 22.57</td>
<td>29.60 ± 17.92</td>
<td>13.69 ± 11.40</td>
<td>15.95 ± 14.97</td>
<td>5.91 ± 10.70</td>
<td>11.01 ± 14.10a</td>
</tr>
<tr>
<td>&gt;40</td>
<td>27</td>
<td>30.78 ± 23.21</td>
<td>33.20 ± 23.36</td>
<td>15.06 ± 10.49</td>
<td>15.49 ± 12.17</td>
<td>4.12 ± 5.95</td>
<td>11.08 ± 14.17a</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>35.02 ± 23.70</td>
<td>31.04 ± 22.28</td>
<td>13.23 ± 10.84</td>
<td>15.25 ± 13.89</td>
<td>5.17 ± 8.56</td>
<td>14.90 ± 17.15a</td>
</tr>
</tbody>
</table>

Values are mean ± SD.

abValues (two patient values were missing) in the same column with different superscripts are significantly different (P < 0.05).

GV: oocytes in the germinal vesicle stage; GVBD: oocytes in germinal vesicle breakdown.

Oocyte morphology and ratios of oestradiol:testosterone and oestradiol:progesterone

The ratio of oestradiol:testosterone was significantly associated with the proportion of metaphase II oocytes at germinal vesicle and GVBD stages and oocytes with cytoplasmic inclusions. When the oestradiol:testosterone ratio was in the range 0–100, a smaller proportion of immature oocytes at the germinal vesicle and GVBD stages (9%) was obtained compared with the proportion at the other oestradiol:testosterone ratios (P < 0.05, Table 4). A greater proportion of oocytes with cytoplasmic inclusions (22%) was observed when the oestradiol:testosterone ratio was in the range 0–100 (P < 0.05, Table 4). When the oestradiol:testosterone ratio was > 200, a significantly smaller proportion (20%) of oocytes at grades 1 and 2 (poor quality) and a higher proportion (42%) of grade 3 and 4 oocytes were obtained. There was no statistical difference when oestradiol:progesterone ratios were compared in terms of metaphase II oocytes at germinal vesicle and GVBD stages (Table 5). However, the proportion of oocytes with cytoplasmic inclusions was significantly higher (26%) when the oestradiol:progesterone ratio was in the range 0–20.
compared with 11% when the oestradiol:progesterone ratio was > 20 (Table 5).

The concentrations of oestradiol, progesterone and testosterone in the follicular fluid were not associated with the proportions of oocytes fertilized and embryo development (data not shown).

Discussion

It has been suggested that the developmental potential of human oocytes is related more closely to the ratio of oestrogen:androgen in follicular fluid than to absolute concentrations of follicular oestrogen (Andersen, 1993). The ratio of oestradiol:androgen in follicular fluid obtained throughout the follicular phase in women with normal menstrual cycles appears to be one of the most precise parameters for distinguishing healthy follicles from atretic follicles with a diameter > 6 mm (Bomsel-Helmreich et al., 1979; McNatty et al., 1979; Westergaard et al., 1986; Seibel et al., 1989). Healthy follicles contain a significantly higher ratio of oestradiol:testosterone than atretic follicles (Fukuda et al., 1995). In women who were smokers, high concentrations of 4-androstene-3,17-dione and testosterone and low concentrations of oestradiol were found in follicles that failed to give rise to cleaved oocytes (Gustafson et al., 1996). De Sutter et al. (1991) found an increased incidence of cytogenetic abnormalities in oocytes retrieved from follicles with an increased ratio of 4-androstene-3,17-dione: oestradiol. In the present study, the proportion of grade 1 and 2 oocytes (poor quality and mainly with an enlarged perivitelline space) was significantly higher when the ratio of oestradiol:testosterone was < 100. This indicates that grade 1 and 2 oocytes originated mostly from the atretic follicles.

Cytoplasmic inclusions, also called refractile body, are composed of multivesicular bodies with lipid droplets, dense granules, small vesicles and fibrillar material. Veeck (1988) reported a strong tendency for oocytes with inclusions to recur in the same patient in repetitive treatment cycles. Xia (1997) demonstrated that the presence of cytoplasmic inclusions was highly correlated with lower fertilization rate and poor embryo quality. Patients greater than 35 years old had a higher proportion of oocytes with cytoplasmic inclusions. It is unclear why such structures occur in the cytoplasm of the oocyte. In the follicle, LH regulates steroidogenesis by stimulating thecal androgen production, and it induces LH receptors and promotes aromatization of androgens to oestradiol in granulosa cells (Zeleznik and Benyo, 1994). Follicular atresia is correlated with a decrease in oestradiol synthesis concomitant with increased progesterone production (Hsu et al., 1994). In the present study, the proportion of oocytes with cytoplasmic inclusions was significantly higher (26%) when the ratio of oestradiol:progesterone was 0–20 compared with 11% when the ratio of oestradiol:progesterone ratio was > 20. Cytoplasmic inclusion rate was also higher when the ratio of oestradiol:testosterone was < 100. These results indicate that oocytes with cytoplasmic inclusions may come from atretic follicles that have low oestradiol concentrations and high progesterone and testosterone concentrations. Whether adjusting stimulation protocols could avoid the occurrence of cytoplasmic inclusions requires further investigation.

In general, there are two different modes of action for the effect of steroids on cells (Tesarik and Mendoza, 1997). The classical (or genomic) mechanism involves a nuclear steroid receptor that acts as a transcription factor (Carson-Junca et al., 1990). The alternative mechanism was identified more recently and does not involve modification of gene activity (non-genomic). Both of these mechanisms appear to be active in human oocytes (Wu et al., 1993; Tesarik and Mendoza, 1995). Tesarik and Mendoza (1997) suggested that oocytes need to be primed with oestrogen to develop Ca²⁺ oscillations during maturation and that this action of oestrogen can be counteracted by androgen through the non-genomic activity. They suggested that in vivo exposure of oocytes to inadequate oestradiol:androgen ratios might result in an abnormal Ca²⁺ response to spermatozoa at fertilization and thus may be responsible for impaired developmental potential of the resulting embryo.

In conclusion, oocyte morphology as assessed by the status of the first polar body and the size of perivitelline space is associated with the ratio of oestradiol:testosterone and the ratio of oestradiol:progesterone but not with the absolute concentrations of oestradiol, progesterone and testosterone in the follicular fluid. An oestradiol:testosterone ratio > 200 is associated with a small proportion of grade 1 and 2 oocytes (poor quality), a large proportion of grade 3 and 4 oocytes (good quality), and also a small proportion of oocytes with cytoplasmic inclusions. The proportion of oocytes with cytoplasmic inclusions is also related to an oestradiol:progesterone ratio < 20.

The authors thanks members of the Gamete Biology Laboratory and the Fertility Clinic of the Hamilton Health Sciences Corporation for the provision of samples. The expenses of this investigation were defrayed by grants from the Chedoke-McMaster Hospitals Foundation and the F. L. Johnson Fund at McMaster University.

References


Carson-Junca MA, Schrader WT and O’Malley HW (1990) Steroid receptor family: structure and function Endocrine Review 11 201–220


Follicular steroid concentrations and oocyte morphology in humans


Westergaard L, Christensen IJ and McNatty KP (1986) Steroid levels in ovarian follicular fluid related to follicle size and health status during the normal menstrual cycle in women Human Reproduction 1 227–232


Xia P (1997) Intracytoplasmic sperm injection: correlation of oocyte grade based on polar body, perivitelline space and cytoplasmic inclusions with fertilization rate and embryo quality Human Reproduction 12 1750–1755
