NOTE

Relationship Among the Status of the Human Oocyte, the 17β-Estradiol Concentration in the Antral Fluid and the Follicular Size

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Abstract

We studied the relationship among the status of the human oocytes, the E₂ concentration in the antral fluid and the follicular size in the different phases of the menstrual cycle, in order to determine the microenvironment of the follicles with healthy or degenerative oocytes in the human ovary. In the follicular phase of the menstrual cycle, follicles which contained a healthy but not degenerative oocyte had a significantly higher level of 17β-estradiol (E₂). In the late follicular phase, the larger follicles (≥ 13 mm, in diameter) had only healthy oocytes. It seems that the follicle containing a degenerative oocyte does not develop physiologically until maturation of the preovulatory follicle.

In the luteal phase, there were no relationships among the status of the oocyte, E₂ concentration in the antral fluid and the follicular size. However, the E₂ levels of the antral follicles with healthy oocytes in an ovary with corpus luteum were significantly lower than those in the contralateral ovary. The results suggest that the corpus luteum may exert an influence on the adjacent follicles.

In the human ovary the development or atresia of the antral follicles occurs at various stages of the menstrual cycle. Although the levels of steroids in the antral fluid, the follicular size and their relationship have been described (Sanyal et al., 1974; Bomsel-Helmreich et al., 1979), there have been few reports on the status of the human oocytes correlating with the E₂ concentration in the antral fluid (McNatty et al., 1979).

We studied the microenvironment of the follicles with healthy or degenerative oocytes and the relationship between the status of oocytes and follicular developments. Factors given attention were the follicular size and the level of steroid, particularly the 17β-estradiol (E₂) concentration in the antral fluid. We now report the relationships among the status of the human oocytes, the E₂ concentration in the antral fluid and the follicular size in the different phases of the menstrual cycle.

Materials and Methods

Ovarian specimens were obtained with permission from 66 patients (aged 30-45 yr) who underwent laparotomy for gynecologic surgery. The indications for surgery were myoma of the uterus and cancer of the cervix (Stage 0 and I). These women had regular menstrual cycles and were considered to be endocrinologically normal. The operations were performed in the various phases of the menstrual cycle (28-30 days). The cycle day was estimated from the date of the last menstrual period and the
basal body temperature (BBT) chart. The presence or lack of a corpus luteum at laparotomy and the endometrium were examined. About 40% of these women were in the luteal phase of the cycle. The follicular phase of the cycle in the remaining women was separated into three: early follicular (cycle days, 3–5), mid-follicular (cycle days, 6–9) and late follicular (cycle days, 10–13) phases.

The ovarian samples were examined to ascertain the absence of major ovarian pathology. The ovarian specimens obtained by wedge resection of the ovary or ovariectomy were placed on ice in Ham's F-10 tissue medium. All the visible follicles were dissected from the specimens within 3 hours of the surgery. The diameter was measured with fine calipers.

The antral fluid was gently aspirated through a 18–23 gauge needle and then transferred into a sterile hollow glass and immediately examined under a dissecting microscope. The antral fluid obtained from each follicle was frozen at −20°C until steroid assay. The E_2 concentration in the antral fluid was determined from duplicate samples assayed by RIA techniques (Wu and Lundy, 1971).

The oocytes were classified into two types: healthy and degenerative. Oocytes were scored as healthy if they showed 2 or more layers of surrounding corona radiata cells, spherical shape and homogeneous cytoplasm, and as degenerative if they showed vacuolization, cytolyis, necrosis, loss of spherical shape, or one layer or lacked the surrounding corona radiata cells. When the corona radiata cells obscured the status of the oocyte, the oocyte was classified during the culture, since it could be readily released from the cells. The oocytes were cultured according to the method of McNatty et al. (1979) with slight modification. The diameter of each oocyte was measured with an ocular micrometer.

Mean and standard errors for each group of samples were calculated, and the statistical significance of the data was evaluated by Student’s t-test.

Results

One hundred and forty-nine oocytes were recovered from the 185 follicles. The healthy oocytes constituted 40.7% of all the recovered oocytes in the follicular phase and 48.3% in the luteal phase. The mean diameter of 65 healthy oocytes was 116.1 ± 1.5 (SE) μm and that of 84 degenerative oocytes was 110.1 ± 1.3 μm (p < 0.01).

Healthy and degenerative oocytes in the follicular phase were examined to determine the relationship between the follicular size and the E_2 concentration in the follicles (Table 1). The E_2 concentration in the follicles rose as follicle size increased. The E_2 concentration in follicles (≤4 mm, in diameter) containing healthy oocytes was higher than that of the same sized follicles which contained degenerative oocytes. In 5–8 mm and 9–12 mm follicles, the E_2 concentration showed the same tendency, and in the late phase the difference was significant (p < 0.05). In the 9–12 mm follicles, healthy oocytes were absent in the early phase, but in the largest follicles (>13 mm, in diameter) all the oocytes were healthy and the E_2 concentration in the antral fluid was about three times higher than that in the 9–12 mm follicles in the late phase (p < 0.001).

<table>
<thead>
<tr>
<th>Follicular phases</th>
<th>Status of oocytes</th>
<th>Follicular diameter (mm)</th>
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<th>Follicular diameter (mm)</th>
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<tbody>
<tr>
<td></td>
<td>Healthy oocyte</td>
<td>≤4</td>
<td>5–8</td>
<td>9–12</td>
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<tr>
<td>Early phase</td>
<td></td>
<td>51.9 ± 7.1 (7)</td>
<td>103.5 ± 21.9 (6)</td>
<td>−</td>
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<tr>
<td></td>
<td>Degenerative oocyte</td>
<td>8.1 ± 2.3 (5)</td>
<td>12.6 ± 5.3 (9)</td>
<td>174.8 ± 52.3 (6)</td>
</tr>
<tr>
<td></td>
<td>Healthy oocyte</td>
<td>73.7 (1)</td>
<td>352.3 ± 86.5* (6)</td>
<td>563.3 ± 127.8*** (8)</td>
</tr>
<tr>
<td>Late phase</td>
<td>Degenerative oocyte</td>
<td>11.2 ± 2.2 (8)</td>
<td>20.0 ± 10.0* (17)</td>
<td>183.2 ± 67.0** (9)</td>
</tr>
</tbody>
</table>

1) Values are means ± S.E.M. in ng/ml.
2) Number of observations is in parenthesis.
3) *; **; Statistical significance, p < 0.05. ***; Statistical significance, p < 0.001.
The relationship between the follicular size and the E2 concentration in the antral fluid and the status of the oocyte in the luteal phase was also investigated (Table 2). The E2 concentration in the follicles containing healthy or degenerative oocytes remained low despite an increase in follicle size, when compared with the follicles associated with the healthy oocytes in the follicular phase.

The corpus luteum was also investigated for a possible influence on the E2 concentration in the follicular fluid (Table 3). In the degenerative oocyte group, there was no difference in E2 concentrations between the follicles from ovaries with or without a corpus luteum. However, in the healthy oocyte group, the E2 concentration in the antral follicles from an ovary with a corpus luteum was significantly lower than that from an ovary without a corpus luteum (p<0.01).

Discussion

As degenerative human oocytes show various visible signs of degeneration under the microscope, we classified these tissues not on the basis of function (maturation or fertilization) but status (healthy or degenerative). Each type in our study essentially appeared at same rate as noted by Sanyal et al. (1976).

Antral fluid and granulosa cells seem to contribute to the maintenance of healthy oocytes in an avascular region. An adequate number of granulosa cells associated with the high rate of mitosis is necessary for the maintenance of viable oocytes (McNatty et al., 1979). On the other hand, a certain level of E2 concentration in the follicles seems to be essential for the granulosa cell mitosis to develop follicles further. Therefore, the E2 concentration in the antral fluid is considered to be a factor in the hormonal environment for oocytes and follicular development.

In our study the degenerative oocytes were never over 13 mm in diameter even in the late follicular phase. Among the healthy oocytes, the E2 concentration in follicles with a diameter larger than 13 mm was significantly higher than those with a diameter less than 9–12 mm (p<0.001), so it seems that only the former is capable of ovulating. As the preovulatory follicular size is usually 18 mm and over (McNatty et al., 1979), not the degenerative oocytes but the healthy ones reach ovulation. The status of the oocyte...
proved to be a useful in determining the characteristics of the follicles in the follicular phase.

In the luteal phase, the E2 concentration in the antral fluid was generally lower than that in the follicular phase. No difference in E2 concentration in the antral fluid was found when the healthy and degenerative oocytes were compared. Nor was there any difference in E2 concentration among the various follicular sizes. However, the E2 concentration in follicles containing healthy oocytes with a corpus luteum was significantly different from the concentration in follicles containing healthy oocytes without a corpus luteum. The substances in the corpus luteum exerting an influence on the adjacent follicles remain to be determined.

References


