Relationship between endometrial histology, morphometry and ultrasound texture in the follicular phase of infertile women with natural menstrual cycles

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The relationship between endometrial histology and ultrasound texture in the follicular phase was investigated. The endometrial sonographic texture of 32 infertile women with normal menstrual cycles was classified into three patterns (L, H and I) and histological and morphometrical analyses were performed. Endometrial specimens from pattern L, which showed multi-layered endometrium characterized by three hyperechogenic lines with inner hypoechogenic regions, had smaller, similar-sized endometrial glands and few stromal cells. Those from pattern H which showed entirely homogeneous hyperechogenic endometrium, had larger, various-sized glands and more stromal cells. Those from pattern I which showed heterogeneous hyperechogenic and partially hypoechogenic endometrium, had the largest, most variable-sized glands and many stromal cells. The differences in sonographic texture may be related to the histological and morphometrical findings.

Key words: endometrium/histology/late follicular phase/morphometry/transvaginal ultrasonography

Introduction

There is no doubt that uterine endometrium has an important role for implantation of embryo(s) at early stages of development in reproductive physiology. Since the histological study of Noyes et al. (1950), endometrial changes have commanded public attention. Uterine endometrium shows dynamic and cyclic morphological changes throughout the follicular phase and into the secretory phase (Lenz and Lindenberg, 1990; Bakos et al., 1993). With the introduction of the vaginal ultrasound transducer, some of these changes can be followed macroscopically and non-invasively. In natural menstrual cycles, the most typical ultrasound findings are of multi-layered endometrium in the late follicular phase which is entirely homogeneous and hyperechogenic in the mid-luteal phase (Bakos et al., 1993). However, other patterns have already been detected from the follicular phase (Sher et al., 1991).

Several authors have demonstrated that the sonographic pattern of the endometrium before human chorionic gonadotrophin (HCG) injection during in-vitro fertilization (IVF) cycles could predict the subsequent occurrence of pregnancy, in particular a multi-layered endometrium pattern being associated with a good conception rate (Gonen and Casper, 1990; Sher et al., 1991; Chech et al., 1993; Coulam et al., 1994). This suggests that in the follicular phase, the endometrium already has some factors affecting embryo implantation, and those factors are reflected in the sonographic pattern. However, to date, histological differences in relation to the sonographic pattern have not been clearly established.

In the present study, we attempted to clarify the difference in endometrium in the follicular phase in relation to histological, morphological, and hormonal analysis. Morphometrical analysis of the endometrium yielded more objective and detailed information for morphological study.

Materials and methods

A total of 130 infertile women visited the department of Obstetrics and Gynecology, University of the Ryukyus from January–October 1992. For infertility work-up we followed one natural cycle from the previous late secretory phase to the next period in all cases. From those women, 32 with normal ovulation who visited our hospital regularly and agreed to endometrial biopsy were enrolled on this study. Of those 32 women, 18 suffered from primary and 14 from secondary infertility. Backgrounds and details of conditions in the three patient groups are given in Table I. The mean age (± SD) of the women was 35.7 ± 5.1 years (range 24–44 years) and the mean (± SD) infertile period was 6.0 ± 3.4 years (range 2–16 years). Previous parity is shown in Table II. For monitoring follicular and endometrial development, transvaginal ultrasonography (Toshiba SSA-90A, 5 MHz, Toshiba Medical Systems, Tokyo, Japan) was performed serally from the previous late secretory phase to the next period. In the late follicular phase, the endometrial sonographic pattern was classified (by two observers) as follows: (i) pattern L (leaf-like pattern), a multi-layered endometrium characterized by three hyperechogenic lines with inner hypoechogenic regions (Figure 1A), (ii) pattern H (hyperechogenic pattern), an entirely homogeneous hyperechogenic endometrium (Figure 1B); and pattern I (irregular pattern), a heterogeneous hyperechogenic and partially hypoechogenic endometrium (Figure 1C). A total of eight patients had fibroids four in the pattern L group, three in the pattern H group and one in the pattern I group.

An endometrial biopsy was performed from the anterior and posterior walls of the uterine cavity after assessment of the endometrial pattern. Tissues were fixed immediately in 10% buffered formalin, embedded in paraffin, sectioned, and stained with haematoxylin and
Three microscopic sample fields were selected arbitrarily for each.

Parameters were measured: (i) area of each gland in the sample field ($\mu m^2$); (ii) area of the sample field ($nm^2$); (iii) total area of nuclei of stromal cells (Jim et al., 1988). With this set-up, the following automated analysis was carried out using a computer-assisted image analyser (Nexus 6400; Nexus, Osaka, Japan) attached to a light microscope and high resolution colour video camera (details of this system as in Tohyama et al., 1988). From these parameters we calculated the mean area of one gland ($\mu m^2$) and the mean number of stromal cells per mm$^2$.

The histological analysis showed that endometrial specimens distributed homogeneously, and few stromal cells (Figure 2A). Those with pattern H had larger and variously-sized glands and the most stromal cells (Figure 2B). Those with pattern I

<table>
<thead>
<tr>
<th>Patient group by sonographic pattern$^*$</th>
<th>No patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary infertility</td>
</tr>
<tr>
<td>Pattern L (n = 16)</td>
<td>9</td>
</tr>
<tr>
<td>Pattern H (n = 7)</td>
<td>4</td>
</tr>
<tr>
<td>Pattern I (n = 9)</td>
<td>5</td>
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</tbody>
</table>

Statistically there was also no significant difference between the groups. Table VI shows serum hormone concentrations on the day of endobiopsy. Statistically there were no significant differences; however, the oestradiol to progesterone ratio and serum LH concentration of the pattern H group appeared to be lower than in the other groups.

### Table I. Backgrounds of the patients in the three groups (mean ± SD)

<table>
<thead>
<tr>
<th>Patient group by sonographic pattern$^*$</th>
<th>No patients</th>
<th>Infertility diagnoses</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary infertility</td>
<td>Secondary infertility</td>
</tr>
<tr>
<td>Pattern L (n = 16)</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Pattern H (n = 7)</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Pattern I (n = 9)</td>
<td>6</td>
<td>1</td>
</tr>
</tbody>
</table>

Statistical analysis

Data were compared by Student's t-test.

**Results**

Of 32 women, 16 demonstrated pattern L, seven pattern H, and nine pattern I. Patient background, maximum size of the pre-ovulatory follicle and endometrial thickness were summarized in Tables III and IV. There were no significant differences between the groups. Table V shows the day of ovulation and the interval between the day of endobiopsy and ovulation.

Statistical analysis

Data were compared by Student's $t$-test.
had the largest, variously-sized glands with many stromal cells (Figure 2C). In order to confirm these findings quantitatively, computer-assisted morphometrical analysis was performed. Morphometrical results are shown in Figures 3 and 4. The mean areas of a single gland ($\times 100^2$ $\mu m^2$) were 5.6 ± 4.7, 7.7 ± 5.2 and 8.9 ± 6.1 for patterns L, H and I respectively. The mean numbers of endometrial stromal cells per mm$^2$ were 5220 ± 1180, 8460 ± 1030, and 7480 ± 2030 for patterns L, H and I respectively. There were significant differences in both measurements between pattern L and patterns H and I. The SD of these parameters were largest in pattern I group in comparison with the others.

Discussion

Since the introduction of IVF and embryo transfer for treatment of infertile women, the fertilization rate and development of fertilized oocytes have significantly increased around the world. However, the implantation rate per embryo transfer remains stable at only ~20% (American Fertility Society, 1994). The process of embryo implantation holds the key to the improvement of the pregnancy rate following IVF/embryo transfer treatment. Therefore, it is necessary to clarify the nature of endometrium during embryo implantation.

Endometrial development and cyclic change are easily observed by transvaginal sonography. In the present study, three types of endometrial texture could be defined (patterns L, H and I) in the follicular phase of infertile women with normal menstrual cycles using transvaginal sonography. The same endometrial pattern persisted throughout the follicular phase but increased dramatically in the late secretory phase (from LH surge + 2 to LH + 6 days) where the number of stromal cells increased. Also in this period, endometrial sonographic texture typically turned from being multi-layered into an entirely homogeneous hyperechogenic pattern. In this context, the present study revealed that sonographic texture in the follicular phase might also reflect the number of stromal cells.

Significant differences were seen in the number of endometrial stromal cells between endometrial specimens of pattern L and patterns H and I. The samples from patterns H and I had more stromal cells. Dockery et al. (1990) reported that in the secretory phase (from LH surge + 2 to LH + 6 days) the number of stromal cells increased. Also in this period, endometrial sonographic texture typically turned from being multi-layered into an entirely homogeneous hyperechogenic pattern. In this context, the present study revealed that sonographic texture in the follicular phase might also reflect the number of stromal cells.

An immunohistological study reported that lymphocytes in endometrial stroma occupied only 8% of stromal cells in the follicular phase and increased dramatically in the late secretory phase (Bulmer et al., 1991). In the present study we only calculated the number of the stromal cells. Therefore we cannot determine what types of cell increased in the endometrial stroma of patterns H and I. This is an interesting problem considering the relationship between embryo implantation and immunology. The reasons why these morphological differences occurred are not known. The interval between the day of endobiopsy and ovulation in the pattern H group appeared to be longer than in pattern L and I groups (difference not significant). In addition, the serum LH concentration was lowest in the pattern H group. The possibility that serum LH might affect histological findings and increase the number of endometrial stromal cells was not borne out by our results, since pattern H with the lowest serum LH concentration had the highest number of stromal cells.

Ohno et al. (1995) mentions that induction of high numbers of progesterone receptor (PR) in the pre-ovulatory period may be related to adequate endometrial growth and increase the responsiveness of the endometrium to progesterone stimulation after ovulation. This suggestion is interesting and the differences in sonographic texture might be related to PR.

There were no differences in serum oestradiol and progester-
Figure 1. Sonographic texture in the late follicular phase. (A) pattern L: a multi-layered endometrium characterized by three hyperechogenic lines with inner hypo-echogenic regions; (B) pattern H: an entirely homogeneous hyperechogenic endometrium, (C) pattern I: a heterogeneous hyperechogenic and partially hypo-echogenic endometrium.

Figure 2. Histological findings in endometrial biopsies from the late follicular phase. (A) Pattern L: small and similar-sized glands were homogeneously distributed and there were few stromal cells; (B) pattern H: glandular size was larger and more variable with the highest number of stromal cells; (C) pattern I: glandular size was largest and variable with a high number of stromal cells.

one concentrations. Johannisson et al. (1982) reported that the glandular diameter was positively correlated with follicular phase oestradiol concentration. However in the present study there was no significant difference in serum oestradiol between the three groups. Even though serum oestradiol concentrations affect glandular size, the variation in glandular size could not be completely explained. However, the oestradiol/progesterone ratio in pattern H appeared to be lower (not significant) than for the other patterns and this parameter might relate to our histological findings.

The clinical finding of some cases with pattern H and I having thick (≥5 mm) endometrium during early follicular phase (days 3–5) was suggestive (data not shown). We suspect that such cases were generated by inadequate shedding of menstrual endometrium, which might in turn affect subsequent endometrial growth and sonographic texture. In the late secret-
pattern corresponding to pattern L and endometrial thickness in natural cycles improve to 'optimal' grade (i.e. a 'halo grades (including homogeneous hyperechogenic endometrium, and reported that women with 'poor' endometrial phase according to both the thickness and echogenicity of the endometrial aspiration (Sakumoto et al, 1992).

In conclusion, our study has demonstrated that differences in sonographic texture were related to histological and morphometrical findings. Further studies are needed to confirm the interpretation which is suggested by these morphological differences.

References


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![Figure 3. The size of a single endometrial gland in the late follicular phase (mean ± SD) according to sonographic pattern (see Table I for explanation)](image1)

![Figure 4. The number of endometrial stromal cells per mm² (mean ± SD) according to sonographic pattern (see Table I for explanation)](image2)

Figure 4. The number of endometrial stromal cells per mm² (mean ± SD) according to sonographic pattern (see Table I for explanation)