Transvaginal ultrasound studies of vascular and morphological changes in uteri exposed to diethylstilbestrol in utero

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The aim of this prospective study was to establish complementary data of uteri exposed to diethylstilbestrol (DES) in utero for transvaginal analysis and vascularity changes during the menstrual cycle. A total of 28 women with DES-exposed uteri were compared with 60 non-exposed women. Transvaginal ultrasound and color Doppler imaging were performed on days 5 and 22 of the menstrual cycle. Uteri were measured on sagittal and transverse scans. Uterine length, width, thickness and uterine cavity length and width were measured. Uterine volume and uterine cavity area were calculated. DES-exposed uterine volume was equal to 31.84 ± 3.37 cm³. The cavity area of DES-exposed uterus was equal to 35.85 ± 3.93 cm². Cervix length of DES-exposed uterus was significantly smaller than that of non-exposed uterus. The uterine artery pulsatility index (PI) of DES-exposed uterus was significantly higher than that of normal uterus. Blood flow remained stable throughout the menstrual cycle. The PI of DES-exposed uterus remained stable during the menstrual cycle, as in non-exposed uterus, and it decreased during the luteal phase. This lack of modification in vascularity of DES-exposed uterus may explain miscarriages and obstetric complications such as intrauterine growth retardation or pre-eclampsia. The data may have implications for the assessment of reproductive status and the design of future studies on disorders of implantation in DES-exposed uterus.

Key words: colour Doppler imaging/DES-exposed uterus/transvaginal ultrasonography/uterine arteries

Introduction

Patients exposed to diethylstilbestrol (DES) in utero have long-term problems with their reproductive organs (Smith et al., 1949). Although prescription was prohibited in the USA after the publication of papers by Herbst et al. (Herbst and Scully, 1970; Herbst et al., 1971, 1974; Herbst, 1979), an estimated 4 000 000 women had already taken the drug.

The main complication of exposure to DES is uterine malformation. The frequency of uterine malformation has been estimated by Kaufmann et al. (1977) to be 73%. The risk does not depend on the total dose absorbed but on how early was exposure to DES. Several control case studies have been published to assess the effect of uterine malformations (Ksenekjian, 1988) on the fertility of women exposed in utero to DES. Some studies have indicated that a reduction in fertility is statistically significant. Nevertheless, 80% of women exposed in utero to DES wanting a child became pregnant and gave birth to a viable baby (Sandberg, 1981; Kaufman et al., 1984). However, Herbst et al. (1982) reported a higher percentage of premature births, ectopic pregnancies and in-utero fetal deaths in the DES group. Spontaneous miscarriages and late abortions were found to be significantly higher in the DES group by Ludmir et al. (1987) and Senekjian (1988). The work of Herbst et al. (1981) has been confirmed by many authors (Berger and Goldstein, 1980).

Transvaginal sonography is the least invasive technique used to assess the uterus and monitor modifications in endometrial thickness during the cycle. The proximity of the probe and the use of high frequencies generate echographic images with excellent definition. Colour Doppler imaging and pulsed Doppler spectral analysis provide data on blood flow in the uterine and sub-endometrial arteries. Vascular indices decrease during the menstrual cycle (Zaidi et al., 1995). An increase in vascularity is necessary for good uterine receptivity (Steer et al., 1992).

Our aims were to obtain complementary data on the morphology and vascularization of DES-exposed uteri using transvaginal sonography and to evaluate vascularization of DES-exposed uteri during the menstrual cycle.

Materials and methods

Object

A total of 28 patients exposed to DES in utero agreed to participate in this study. Some 60 patients with primary or secondary infertility also joined this study as controls. The 60 control patients were not exposed in utero to DES. The DES and control patients had regular, ovulating, menstrual cycles confirmed by a temperature graph. None of these patients had been given any hormonal treatment over the previous 6 months.

Pelvic ultrasound

An Ultra Mark 9 (ATL) echograph (Advanced Technology Laboratory, Bothell, USA) equipped with a transvaginal probe in mode B with a colour Doppler mode was used for all ultrasound examinations. The vaginal probe was equipped with a 5 MHz transducer for mode B and the Colour Doppler functions.

Transverse and sagittal sections were used for all uteri. Maximum

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Table I. Characteristics of the two patient groups exposed and non-exposed (values are means ± SD)

<table>
<thead>
<tr>
<th></th>
<th>DES-exposed uteri (n = 28)</th>
<th>Normal uteri (n = 60)</th>
<th>P (Student's t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>30.4 ± 1.8</td>
<td>29.7 ± 0.6</td>
<td>NS</td>
</tr>
<tr>
<td>No. of miscarriages</td>
<td>0.65 ± 0.21</td>
<td>0.08 ± 0.03</td>
<td>0.05</td>
</tr>
<tr>
<td>Parity</td>
<td>0.60 ± 0.16</td>
<td>0.56 ± 0.13</td>
<td>NS</td>
</tr>
<tr>
<td>No. of gestations</td>
<td>1.56 ± 0.38</td>
<td>0.34 ± 0.14</td>
<td>0.05</td>
</tr>
<tr>
<td>Day of the first ultrasound (follicular phase)</td>
<td>6.48 ± 0.70</td>
<td>5.60 ± 0.15</td>
<td>NS</td>
</tr>
<tr>
<td>Day of the second ultrasound (luteal phase)</td>
<td>22.60 ± 1.41</td>
<td>25.00 ± 0.18</td>
<td>NS</td>
</tr>
<tr>
<td>Oestradiol concentrations (pg/ml)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day of the first ultrasound</td>
<td>62.69 ± 9.20</td>
<td>51.53 ± 2.43</td>
<td>NS</td>
</tr>
<tr>
<td>Day of the second ultrasound</td>
<td>16.83 ± 31.40</td>
<td>141.21 ± 5.31</td>
<td>NS</td>
</tr>
</tbody>
</table>

DES = diethylstilbestrol; NS = not significant

Table II. Transvaginal measures of exposed and non-exposed uterus

<table>
<thead>
<tr>
<th></th>
<th>DES-exposed uteri (n = 28)</th>
<th>Normal uteri (n = 60)</th>
<th>P (Student's t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine length (mm)</td>
<td>42.77 ± 1.80</td>
<td>54.90 ± 0.92</td>
<td>0.0001</td>
</tr>
<tr>
<td>Uterine width (mm)</td>
<td>44.92 ± 1.402</td>
<td>51.36 ± 1.08</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine thickness (mm)</td>
<td>29.28 ± 1.13</td>
<td>36.93 ± 0.85</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine volume (cm³)</td>
<td>31.84 ± 3.37</td>
<td>56.17 ± 2.72</td>
<td>0.001</td>
</tr>
<tr>
<td>Cavity length (cm)</td>
<td>32.22 ± 1.61</td>
<td>42.21 ± 1.68</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cavity width (mm)</td>
<td>21.36 ± 1.68</td>
<td>23.50 ± 0.94</td>
<td>NS</td>
</tr>
<tr>
<td>Cavity surface (cm²)</td>
<td>35.85 ± 3.93</td>
<td>50.58 ± 2.45</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cervix length (mm)</td>
<td>23.60 ± 1.19</td>
<td>28.86 ± 0.86</td>
<td>0.01</td>
</tr>
<tr>
<td>Endometrial thickness (mm)</td>
<td>3.85 ± 0.45</td>
<td>4.98 ± 0.22</td>
<td>0.05</td>
</tr>
<tr>
<td>Follicular phase</td>
<td>8.27 ± 0.74</td>
<td>11.01 ± 0.37</td>
<td>0.01</td>
</tr>
<tr>
<td>Luteal phase</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

DES = diethylstilbestrol; NS = not significant.

uterine length (L; cm) was measured on a sagittal section (cm) from the internal cervical os to the fundus. Uterine width (W; cm) was calculated on a transverse section passing through the uterine fundus. Uterine thickness (T) was always measured by a sagittal section (cm) between the anterior and posterior walls. Uterine volume (cm³) was estimated using the formula of Viscomi et al. (1980) (4/3π(L/2×W/H/2×W²)). By tilting the end of the vaginal probe to the rear, cervical length was measured as the distance separating the external and internal os.

The length of the uterine cavity (cm) was assessed by a sagittal section of the uterus, from the internal os to the distal extremity of the endometrium. Endometrial thickness was measured from this section from one endometrium–myometrium interface to the other. Cavity width was measured as the maximum distance between the two extremities of the endometrium from transverse sections passing through the fundus. Uterine cavity surface area was estimated using the ratio L × 1/2. Uterine measurements were always made during the follicular phase, on day 5, except for endometrial thickness which was calculated during two ultrasound examinations on days 5 and 22.

Colour Doppler mode was used to identify the uterine artery as it ascends just lateral to the internal os. The right and left branches of the uterine artery were studied at points where the angle of the ultrasound beam approached zero. The Doppler gate was placed over each vessel to generate a flow velocity with the highest possible peak systolic velocity. The anterior and posterior arcuate arteries were located on a longitudinal section halfway between the uterine edge and the endometrium. Blood flow impedance was expressed by the pulsatility index (PI) to allow for the possible absence of diastolic flow. PI was calculated electronically over three cardiac cycles using the formula PI = S - D/TMAX, where S is the peak systolic Doppler shift frequency, D is the minimum diastolic Doppler shifted frequency and TMAX is the time average maximum velocity over the cardiac cycle. A reduction in the PI is thought to reflect a decrease in impedance distal to the point of sampling. We also examined the mean of the indices for the right and left uterine arteries and the mean of the indices for the anterior and posterior arcuate arteries, as described by Kurjak et al. (1991) and Steer et al. (1990). The Doppler study was carried out on every patient on days 5 and 22 of the cycle.

Hormonal assays

Serum concentrations of oestradiol were measured on examination days (days 5 and 22) by immunoenzymatic assays (E₂ MEIA - IMX; Abbott, Paris France). The echographies were executed at 08:00 h and hormonal assays were at 08:30 h. Serum concentrations of progesterone was not measured on day 22 All assays were based on the temperature graph. Menstrual cycles were considered to be normal if all retrospective criteria were fulfilled, i.e. 25–28 day cycles, shifts in upper temperatures >4/10ths and a significant increase in oestradiol concentration.

Statistical analysis

The statistical analysis was performed using Student's t-test. The level of statistical significance was indicated by P < 0.005.

Results

Table I summarizes the characteristics for patients exposed and not exposed to DES in utero. Both groups were similar in age and oestradiol concentration. Echographies were carried out on the same day in both groups. The duration of infertility, the number of gestations and the number of miscarriages were
Studies in uteri exposed to diethylstilbestrol

Uterine morphology
Details of uterine measurements are summarized in Table II. Uterine cavity length was statistically lower in the group exposed to DES ($P < 0.0001$). Uterine cavity width was not significantly different between the two groups ($P = 0.24$). Uterine cavity surface area of uteri exposed to DES was significantly smaller ($35.8 \pm 3.93$ versus $50.5 \pm 0.9$ cm$^2$; $P < 0.0001$). All dimensions (length, width, thickness, volume) of uterine corpus exposed to DES were significantly smaller than unexposed uteri ($P < 0.0001$). The length of the cervix of uteri exposed to DES was significantly shorter than that of control uteri ($P < 0.05$). However, this difference was only $3$ mm ($25.60 \pm 1.19$ versus $28.80 \pm 0.86$ mm). The endometrial thickness of DES-exposed uteri was significantly smaller in both the follicular and luteal phases. The difference was greater during the luteal phase ($P < 0.001$) than in the follicular phase ($P < 0.01$).

Uterine and arcuate arterial blood flow
Average values of uterine and arcuate artery PI are summarized in Table III. The uterine (Figure 3) and arcuate artery PI of unexposed uteri tended to decrease significantly in the luteal phase ($P < 0.001$). The uterine and arcuate artery PI of uteri exposed to DES in utero (Figure 3) remained stable throughout the cycle ($P = 0.32$).

The uterine PI of DES-exposed uteri was significantly higher than that of control uteri in both the luteal and follicular phases.

Figure 1. Sagittal image of the uterus showing the different measurements taken (day 5 of the menstrual cycle). Measurement 1 = maximum uterine length; measurement 2 = maximum uterine thickness; measurement 3 = maximum uterine cavity length.

Figure 2. Transverse image of the uterus showing the different measurements taken (day 5 of the menstrual cycle). Measurement 1 = maximum uterine width; measurement 2 = maximum uterine cavity width.

Parity was similar in both groups of patients ($P > 0.05$). Eight patients exposed to DES in utero had already had one or several miscarriages. Figure 1 is an example of a transverse section of the uterus and the measurements carried out on this section, i.e. uterine length, cavity length, uterine and endometrial thickness. Figure 2 is an example of a transverse section through the fundus and measurements of uterine cavity and uterine widths.
Table III. Mean value of the pulsatility index (PI) in uterine and arcuate arteries in exposed and non-exposed uterus

<table>
<thead>
<tr>
<th>Pl</th>
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<th>Normal uterus (n = 60)</th>
<th>P (Student's t-test)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uterine follicular phase</td>
<td>3.12 ± 0.22</td>
<td>2.49 ± 0.05</td>
<td>0.001</td>
</tr>
<tr>
<td>Uterine luteal phase</td>
<td>3.06 ± 0.23</td>
<td>1.93 ± 0.05</td>
<td>0.0001</td>
</tr>
<tr>
<td>Arcuate artery</td>
<td>1.97 ± 0.17</td>
<td>1.67 ± 0.05</td>
<td>0.06</td>
</tr>
<tr>
<td>Arcuate artery luteal phase</td>
<td>2.03 ± 0.19</td>
<td>1.43 ± 0.06</td>
<td>0.001</td>
</tr>
</tbody>
</table>

(P < 0.001). For arcuate arteries, the PI are similar for both DES-exposed and non-exposed groups during the follicular phase (P = 0.21, not significant). In the luteal phase, the arcuate PI of DES-exposed uteri remained stable compared with the follicular phase (1.97 ± 0.17 versus 1.40 ± 0.06), whereas the arcuate PI of unexposed uteri decreased. In this case the difference was significant (P < 0.01).

**Discussion**

DES is known to affect the aspect and size of uteri (Kaufmann et al., 1984). Many non-neoplastic malformations have been described (Goldstein, 1978). Kaufmann et al. (1986) have reported uterine cavity anomalies in 61.5% of patients exposed to DES. However, all studies that have reported on uterine anomalies have used X-rays. Uterine hypoplasia is the most frequently cited (Thorp et al., 1990) anomaly in the DES syndrome. It is defined as a distance between the two uterine horns of <4 cm, associated with an inversion of the uterine corpus area to uterine neck area ratio. According to Cousins (1980), 44% of patients exposed to DES in utero had hypoplastic uteri. Other malformations include anomalies of uterine shape, the isthmus and the uterine horns (Levine and Erkowitz, 1993).

Visconi et al. (1980) are the only authors to have reported an ultrasound study of uteri exposed to DES. This study was carried out by transabdominal sonography. The difference between control and DES-exposed uterine volume was 50%. The mean uterine volume of the control group was 90 cm³, versus 49.4 cm³ for DES-exposed uteri. Transvaginal ultrasound has completely modified the investigation of the uterus. The proximity of the probe to the investigated organs and the possibility of using high frequency transducers permit high-definition imaging to be obtained. Transvaginal sonography has enabled an analysis of the uterus in two dimensions, sagittal and transversal. Measurements of the uterus are much more accurate than those obtained by the abdominal approach. Transvaginal sonography also enables an investigation of the endometrium and its modifications during the menstrual cycle. Our study confirmed existing data. The dimensions of DES-exposed uteri are significantly reduced compared with unexposed uteri. This reduction affects not only uterine corpus volume but also endometrial surface area. The endometrial surface area of DES-exposed uter was reduced simply because the length of the endometrial cavity was shorter. This reduction in endometrium surface area may explain smaller menstrual blood volume. In 1994, Hornsby et al. confirmed that the duration and volume of menstrual bleeding in women exposed to DES was reduced.

Endometrial modifications are essential for successful embryo implantation (Applebaum, 1995). The combination of transvaginal sonography and pulsed colour Doppler is now used by many teams (Kurjak et al., 1991; Bied et al., 1995; Tekay et al., 1995) to obtain additional data about endometrial receptivity by studying endometrial and haemodynamic modifications of the uterus. In in-vitro fertilization, a PI value >3 on the day of embryo transfer is associated with a decreased rate of implantation (Steer et al., 1992). Others (Bassil et al., 1995) believe that if the PI is high at the time of embryo transfer, then transfer should be delayed. Bourne et al. (1996) showed recently...
that during the menstrual cycle the PI of the uterine arteries progressively decreased during the luteal phase. Bourne et al.'s (1996) work confirmed the studies of Steer et al. (1990) and other authors (Scholtes et al., 1989). These data suggest that an appropriate blood flow is required if a potential menstrual cycle is to become a conception cycle after coitus during the fertile period. Implantation failures in women exposed to DES in utero have always been considered to be a result of uterine hypoplasia. The hypothesis is that the uterus cannot be distended during pregnancy, and that failure was purely for mechanical reasons. Uterine pulsatility indices of DES-exposed uteri are significantly higher than those of unexposed uteri. Unlike unexposed uteri, PI values remain >3 in the luteal phase. Blood flow does not increase during the luteal phase despite satisfactory oestrogenic hormone concentrations. Steer et al. (1990) have demonstrated that PI tend to be higher in infertile women. DES can affect uterine vascularization and thus reduce the compliance of uterine vessels. Two hypotheses are possible: DES could introduce a histological anomaly in uterine arteries or uterine arteries may lack sensitivity to oestriol because of a reduction in the number of receptors. The result is a reduction in the blood flow needed to ensure correct implantation. The aetiology of repetitive extremely early miscarriages during pregnancy could be a functional and not a mechanical aetiology, or a combination of the two. Recently, Mittendorf and Williams (1995) reported that pre-eclampsia was twice as frequent in pregnant women exposed to DES than in those not exposed. Mittendorf and Williams (1995) suggested that in-utero exposure to DES induces an anatomical change in the genito-urinary tract with a predisposition to eclampsia. These changes could be located in the uterine artery. Trophoblastic invasion of sub-endometrial arteries could not occur. Our study shows that vascularization of arcuate arteries during the follicular phase is no different in exposed uteri than in unexposed uteri, but that the PI increases during the luteal phase in DES-exposed uteri rather than the unexposed uteri.

Our study confirms previous results and demonstrates that the uteri of women exposed in utero to DES are hypoplastic. Our results also show that the reduction in size of these uteri is global, affecting both the external morphology and the surface area of the endometrial cavity. We also show that modification of the DES-exposed uterus is not only anatomical but involves uterine blood flow. This is particularly helpful in understanding the mechanisms of repeated miscarriage because it suggests that vascular participation may be responsible. The stability of blood flow during the menstrual cycle in Disthène uteri could help to explain repetitive miscarriages. Colour Doppler echography is an interesting addition to hysteroscopy.

Based on these results, we now propose a study by Doppler transvaginal sonography and hysteroscopy for all patients exposed in utero to DES. Colour Doppler echography gives an appreciation of the implantation chances of DES patients. A high PI may in part explain repetitive miscarriages. We suggest low doses of aspirin for these patients from the beginning of pregnancy.

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