Outcome of IVF in DES-Exposed Daughters: Experience in the 90s

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Purpose: The outcome of in vitro fertilization (IVF) in a group of infertile women with a history of in utero exposure to diethylstilbestrol (DES) was analyzed. Records from an academic IVF program were retrospectively reviewed.

Methods: Seventeen infertile women with a self-reported history of exposure to DES in utero, attending the IVF unit at Massachusetts General Hospital (MGH) for assisted reproductive technology (ART), underwent 27 IVF cycles. Analysis of the outcome of IVF including implantation and ongoing pregnancy rates was performed. The data were compared with results from a group of 20 infertile patients with idiopathic infertility undergoing 27 IVF cycles at MGH during the same period. The patients in the two groups were matched for age, basal day 3 levels of follicle stimulating hormone and serum estradiol, and the number and quality of embryos transferred.

Results: The response to controlled ovarian hyperstimulation was comparable in the two groups. Significantly lower implantation and ongoing pregnancy rates following IVF and embryo transfer were seen in the utero DES-exposed group compared to the control patients.

Conclusions: Infertile patients with a history of in utero exposure to DES exhibit a significantly impaired implantation rate following IVF, and the outcome of ART remains poor.

KEY WORDS: diethylstilbestrol; infertility; in vitro fertilization; implantation; pregnancy; assisted reproduction.

INTRODUCTION

Deleterious effects of diethylstilbestrol (DES) on the reproductive outcome are well established. The various factors attributed to impair fertility in women with a history of in utero exposure to DES include a spectrum of uterine, cervical, and vaginal anatomical deformities, shortened menstrual cycles, and altered ovarian steroidogenesis (1–3). Limited data are available regarding the outcome of assisted reproductive techniques (ART) in women with a history of exposure in utero to DES. A review of the literature on the subject of DES exposure and effects on reproductive performance following IVF has yielded only three published studies (4–6). Karande *et al.* (4) have suggested an impaired implantation in this group of patients when compared with cases of tubal infertility. Noyes *et al.* (6), in a comparison of DES-exposed versus nonexposed infertile patients undergoing IVF, have demonstrated statistically insignificant, though clinically significant, differences in implantation rates between the two groups (13.6 vs 23.2%). Since the sequelae of DES exposure were recognized in the 1970s, we anticipate that ART programs, especially in North America, will continue to witness the impact of this teratogen on the reproductive performances of those exposed for at least another decade. Implications of DES use in pregnancy thus continue to pose a challenge to those managing infertility at the turn of the 21st century.

We performed a retrospective analysis of all IVF cycles of patients with a self-reported history of in utero exposure to DES with the objective of assessing the outcome following controlled ovarian hyperstimulation (COH) and embryo transfer. The results were compared to outcome of COH and IVF and embryo transfer in a control group of patients with idiopathic infertility.

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MATERIALS AND METHODS

Seventeen infertile women with a self-reported history of in utero exposure to DES underwent a total of 27 attempts at IVF at MGH between August 1993 and March 1997. Each patient had a basal endocrine profile including a day 3 serum follicle stimulating hormone (FSH) and estradiol (E_2) values checked in an unstimulated cycle. The uterine cavity was evaluated in 5 of 17 patients by a hysterosalpingogram (HSG) and in 12 of 17 by performing hysteroscopy in an office setting. The control group included 20 infertile patients with a diagnosis of idiopathic infertility undergoing IVF cycles during the same period as the DES-exposed population. The diagnosis for the idiopathic group was based on exclusion of all known causes for infertility [i.e., tubal factor, anovulation, male-factor infertility, and endometriosis stages II, III, and IV according to the revised AFS classification (7)]. These patients underwent a total of 27 cycles of IVF and were matched with the DES-exposed group for age, basal FSH and E_2 , and the number and quality of embryos transferred. The uterine cavity was assessed by HSG in 4 of 20 patients and by hysteroscopy in 16 of 20. Pituitary downregulation was employed using gonadotropin releasing hormone agonist (GnRHa) leuprolide acetate (LA; TAP Pharmaceuticals, Deerfield, IL). COH was achieved using human menopausal gonadotropins (Metrodin; Serono Laboratories, Randolph, MA) and hMG (Pergonal; Serono Laboratories, Randolph, MA). Ovarian response was monitored by serial serum E_2 levels and follicular measurements by transvaginal ultrasound scans using a 7.5-MHz vaginal transducer (General Electric, Milwaukee; RT 3200 Advantage II). An assessment of the thickness of the endometrial stripe was performed on the day of hCG administration. Oocyte maturation was induced with 10,000 IU of hCG (Profasi; Serono Laboratories, Randolph, MA), and transvaginal ultrasound-guided oocyte retrieval was performed 34–36 hr after hCG injection. The oocytes retrieved were graded according to the maturity based on the configuration of the corona–cumulus complex (8). The quality of oocytes was determined based on the regularity of the cell and zona pellucida, clarity of cytoplasm, and presence or absence of vacuoles and cytoplasmic fragments, using an internal grading system (unpublished). Quality 1 oocytes showed a regular outline, clear cytoplasm with an intact zona pellucida, quality 2 oocytes showed cytoplasmic vacuoles occupying <20% of the cell volume, and quality 3 oocytes included irregular cells with cytoplasmic fragments and or discoloration. The timing of insemination

varied according to oocyte maturity. Fertilization was checked at 18 hr post insemination and the number of pronuclei assessed under the light microscope using $\times 200$ magnification. Abnormal fertilization was classified as more or fewer than two pronuclei. The embryos were assessed for cleavage status and quality during the next 48–72 hr. The embryos were graded in terms of their shape, the regularity of blastomeres, and the clarity of the cytoplasm and transferred either at 48 or 72 hr after insemination. Three patients in the DES-exposed group had assisted hatching (AH) performed on the embryos following at least two prior failed attempts at IVF. Luteal-phase support was provided with intramuscular progesterone, 50 mg/day. A quantitative serum β -hCG was performed 14 days after embryo transfer; a level >5 IU/L implied a positive test. A chemical pregnancy was defined as a positive serum β -hCG, which subsequently did not rise appropriately or result in a gestational sac on ultrasound scan. A clinical pregnancy was defined as one in which a gestational sac was seen on ultrasound scan. An ongoing pregnancy was a viable pregnancy continuing beyond 20 weeks of gestation. Implantation rate was defined as the number of gestational sacs seen on ultrasound scan per total number of embryos transferred. The clinical pregnancy rate was defined as the number of cycles with fetal sacs on ultrasound scan per number of embryo transfer cycles.

The data were analyzed using paired two-tailed Student's *t* test and Fisher's exact test as appropriate. All data are expressed as mean $\pm$ standard deviation (SD). Statistical significance was defined as $P < 0.05$. The analysis was performed using the SAS statistical package (SAS Institute, Cary, NC).

RESULTS

The patients in both groups were comparable with respect to age and basal FSH and E_2 values (Table I).

Table I. Patient Characteristics: Comparison of DES-Exposed Patients and Controls^a

Patient characteristic	DES	Control
Number of patients	17	20
Age (years)	36.0 $\pm$ 4	34.2 $\pm$ 4
Day 3 FSH(IU/L)	13.4 $\pm$ 5	14.0 $\pm$ 5
Day 3 estradiol (pg/ml)	73.3 $\pm$ 29	59.4 $\pm$ 34
Primary infertility	29% (5/17)	70% (14/20)
Presence of uterine abnormality	94% (16/17)	0% (0/20)
History of ectopic pregnancy	29% (5/17)	0% (0/20)

^a Data are expressed as mean values $\pm$ SD or percentage as appropriate.

Among the DES-exposed patients, normal anatomy of the lower genital tract existed in only 1; the remaining 16 exhibited a spectrum of uterine and/or cervical deformities. The most common uterine anomaly was a T-shaped cavity, present in 7 of 17 cases (41%). In the control group there was no evidence of any abnormality of the uterine cavity. The incidence of primary infertility was higher in the control group (29 vs 70%). Five of the 17 patients with a history of DES exposure in utero had experienced at least one ectopic pregnancy. There was no history of ectopic pregnancy in any of the patients in the control group.

The response of the DES-exposed patients to COH in terms of the maximum serum E₂ levels achieved, the total number of ampoules of gonadotropins used, the timing of hCG administration, and the number of preovulatory oocytes retrieved was comparable to that of the age-matched controls (Table II). The mean thickness of the endometrial stripe on the day of hCG administration in the DES-exposed group was 10 mm, which was also comparable to that of controls. The fertilization rate of 82% and cleavage rate of 81% in the DES-exposed group were also comparable to results for the controls (85 and 85%, respectively).

Fresh embryo transfer was achieved in 24 of 27 cycles in the DES-exposed group. The average number of embryos transferred was three per transfer. Three of the 27 IVF cycles did not result in an embryo transfer secondary to abnormal or failed fertilization. The data from these three cycles are included for analysis of the response to COH as well as to assess the fertilization and cleavage rates for embryos from DES exposed patients. Only the cycles that resulted in embryo transfer are included in the analysis of implantation and

pregnancy rates. All the 27 IVF cycles in the control group resulted in a fresh embryo transfer. The number of embryos transferred, their quality, and the timing of transfer were comparable to those of the DES-exposed group. Assisted hatching was not performed on any of the embryos in this group.

In the DES-exposed patients, a total of three pregnancies resulted from 24 fresh embryo transfer cycles, giving a total pregnancy rate of 12.5% per transfer. One was a chemical pregnancy, one resulted in a live birth at term, and the third is ongoing. The clinical and ongoing pregnancy rate was 8% per transfer for the DES-exposed group. Both the pregnancies attained in the DES-exposed group were singleton, with an implantation rate of 2.3%. In the control group a total of 14 pregnancies resulted from 27 embryo transfer cycles, with a total pregnancy rate of 52%. Of these, two were chemical, six have delivered at term, and the remaining six are ongoing pregnancies. Of the 12 clinical pregnancies in the control group, there were three sets of triplets and a twin pregnancy. The clinical and ongoing pregnancy rate in the controls was 44%. A total of 16 gestation sacs was visualized following the transfer of 101 embryos, giving an implantation rate of 16%. The differences in the implantation rates and the ongoing pregnancy rates between the two groups were statistically significant ($P < 0.05$).

To perform valid comparisons, only the first cycles of IVF were analyzed for patients in each group. The clinical pregnancy rate was 11.8% per transfer in the DES-exposed group versus 40% per transfer in the control group. This difference approached, though did not reach, statistical significance ($P = 0.07$). The implantation rates, considering only the first attempts at IVF, were 4.2 versus 13.7%, respectively, in the DES and control groups ($P = 0.08$).

Table II. IVF Cycles and Their Outcome: Comparison of DES-Exposed Patients and Controls^a

IVF cycles	DES	Control
Total ampoules per cycle	41.0 ± 23	41.0 ± 20
Day of hCG	12.0 ± 2	12.0 ± 1.5
Maximal serum estradiol (pg/ml)	2626 ± 1615	2053 ± 976
Endometrial thickness (mm)	10.0 ± 2	10.0 ± 1
Embryos transferred	3.2 ± 1.8	4.0 ± 1.2
Quality 1 embryos transferred (ET) (%)	15	27
Quality 2 ET (%)	57	46
Quality 3 ET (%)	28	27
Ongoing pregnancy rate/transfer (%)	8.3 (2/24)	44 (12/27) ^b
Implantation rate (%)	2.3 (2/86)	16 (16/101) ^b

^a Values expressed as the mean ± SD or percentage as appropriate.

^b $P < 0.05$, statistically significant.

DISCUSSION

This study confirms a poor outcome of IVF in infertile women with a history of in utero exposure to DES. The significantly impaired implantation rate (2%) following IVF in this category of infertile women is striking and concordant with earlier reported data (4). The results of our analysis also demonstrate that the response to COH remains unaffected by a history of exposure to DES, a finding compatible with earlier reports (4–6). The percentage of postmature or degenerate oocytes retrieved per cycle (3%) was significantly less than the 28.7% quoted by Muasher *et al.* (5) and may reflect improvements in stimulation protocols dur-

ing the past decade. The secondary analysis of data including only first attempts at IVF for patients in each group was undertaken in an effort to eliminate a potential bias introduced by inclusion of multiple results from the same patient. The results of this analysis demonstrated clinically significant impairment in implantation and ongoing pregnancy rates for patients exposed in utero to DES.

The significantly higher predilection to ectopic pregnancy is clearly documented in our study group in whom 29% had a history of at least one tubal pregnancy (Table I). Distorted tubal architecture as well as abnormal physiology has been suggested to contribute to this predisposition to ectopic pregnancy. The risk of ectopic pregnancy is quoted to be eight times greater in women with a history of exposure to DES and appears to be the leading factor contributing to a poor reproductive performance in this group (9).

T-shaped anomaly of the uterine cavity was the most frequently seen anatomic deformity in the DES exposed patients in this study, the incidence being comparable to that reported earlier (4,6). This particular phenotype was associated with a high past incidence of spontaneous miscarriages as well as ectopic pregnancies. No prognostic implication could be attributed to the presence of a T-shaped uterine cavity in terms of ability to conceive and carry a pregnancy to term. Of the two successful IVF cycles in the DES-exposed group, one of the patients had a T-shaped uterine abnormality, while in the second patient the uterine cavity was hypoplastic. The chemical pregnancy following IVF occurred in a patient with a T-shaped cavity. Karande *et al.* (4), in contrast to our conclusions, suggested a poor prognosis for IVF associated with uteri with constrictions and the combination of T-shaped uteri and constrictions.

In the data presented, the embryo cleavage rate and the quality suggest a normal biological potential of embryos in DES-exposed patients. The thickness of the endometrial stripe as measured by ultrasound seems to indicate an appropriate end-organ response to the circulating E_2 , a finding similar to that reported earlier (6). A number of studies (10–14) in the recent past have focused on an association between the thickness and pattern of the endometrial stripe and subsequent implantation; most of the available data imply that an endometrial thickness of less than 7 mm is predictive of a poor outcome. Noyes *et al.* (6) have suggested that the echogenic pattern of the endometrium on TVS is altered in DES-exposed patients with implications for pregnancy outcome following IVF. The mechanism of implantation failure in patients with a history of

DES exposure in utero, however, remains obscure. Salle *et al.* (15) have demonstrated a reduction in uterine blood flow in the luteal phase of menstrual cycle in DES-exposed uteri and have suggested that as a possible cause for impaired implantation in these patients. Information based on an animal model suggests an altered uterine mucosal lining in DES-exposed uteri interfering with embryo attachment to the surface epithelium (16). Castlebaum *et al.* (17) analyzed the expression of integrins in the luteal phase of the endometrium in patients with a history of in utero exposure to DES and showed minimal differences between women with and women without a history of exposure. Assisted hatching has shown some benefit in improving implantation rates in certain subgroups of infertile women undergoing IVF (18). Despite these theoretical benefits of AH, no pregnancy occurred in the three patients with DES exposure who underwent AH of the embryos prior to transfer. None of the embryos transferred in the control group underwent AH. This difference would have biased the data for improved implantation in the DES-exposed patients, which was not seen.

CONCLUSIONS

This study determined impaired implantation to be a major factor contributing to infertility in women undergoing IVF with a history of in utero exposure to DES. The ovarian response to controlled hyperstimulation, the fertilization and cleavage rates, and the embryo quality remain unaffected in these patients. The mechanism for implantation failure remains obscure and the prognosis for ART is guarded for patients exposed to DES in utero.

REFERENCES

1. Peress MR, Tsai CC, Mathur RS, Williamson HO: Hirsutism and menstrual patterns in women exposed to diethylstilbestrol in utero. *Am J Obstet Gynecol* 1982;144:135–139
2. Assies J. Hyperprolactinemia in diethylstilbestrol-exposed women [letter]. *Lancet* 1991;337:983
3. Bibbo M, Gill WB, Azizi F, Blough R, Fang VS, Rosenfield RL, *et al.*: Follow-up study of male and female offspring of DES-exposed mothers. *Obstet Gynecol* 1977;49:1–8
4. Karande VC, Lester RG, Muasher SJ, Jones DL, Acosta AA, Jones HW: Are implantation and pregnancy outcome impaired in diethylstilbestrol exposed women after in vitro fertilization and embryo transfer? *Fertil Steril* 1990;54:No. 2
5. Muasher SJ, Garcia JE, Jones HAW: Experience with diethylstilbestrol-exposed infertile women in a program of in vitro fertilization. *Fertil Steril* 1984;42:No. 1

6. Noyes N, Liu HC, Sultan K, Rozenwaks Z: Endometrial pattern in diethylstilboestrol-exposed women undergoing in-vitro fertilization may be the most significant predictor of pregnancy outcome. *Hum Reprod* 1996;11(12):2719–2723
7. American Fertility Society: Revised American Fertility Society classification for endometriosis. *Fertil Steril* 1985;43:351–352
8. Veeck LL, Wortham JWE Jr, Witmyer J, Sandow BA, Acosta AA, Garcia JE, Jones HW Jr: Maturation and fertilization of morphologically immature human oocytes in a program of in vitro fertilization. *Fertil Steril* 1983;39:594
9. Stillman RJ: In utero exposure to diethylstilbestrol: Adverse effects of the reproductive tract and reproductive performance in male and female offspring. *Am J Obstet Gynecol* 1982;142:No. 7
10. Sandberg EC, Riffle NC, Higdon JV, Getman CE: Pregnancy outcome in women exposed to diethylstilbestrol in utero. *Am J Obstet Gynecol* 1981;140:194
11. Check JH, Nowroozi Km Choe J, Dietterich C: Influence of endometrial thickness and echogenic patterns on pregnancy rates during in vitro fertilization. *Fertil Steril* 1991;56:1173–1175
12. Gonen Y, Casper RF: Prediction of implantation by the sonographic appearance of the endometrium during controlled ovarian stimulation for IVF. *J Vitro Fert Embryo Transfer* 1990;7:146–152
13. Glissant A, de Mouzon J, Frydman R: Ultrasound study of the endometrium during in vitro fertilization cycles. *Fertil Steril* 1985;44:786–790
14. Welker BG, Gembruch U, Diedrich K, Al-Hasani S, Krebs D: Transvaginal sonography of the endometrium during ovum pickup in stimulated cycles for in vitro fertilization. *J Ultrasound Med* 1989;8:549–553
15. Salle B, Sergeant P, Awada A, Bied-Damon V, Gaucherand P, Boisson C, Guibaud S, Benchaib M, Rudigoz RC: Transvaginal ultrasound studies of vascular and morphological changes in uteri exposed to diethylstilbestrol in utero. *Hum Reprod* 1996;11(11):2531–2536
16. Halling A, Von Mecklenburg C, Forsberg JG: Factors of importance for decreased early embryo survival in female mice treated neonatally with diethylstilboestrol. *J Reprod Fertil* 1993;99;2:291–297
17. Castlebaum AJ, Sawin SW, Bellardo LJ, Lessey BA: Endometrial integrin expression in women exposed to diethylstilbestrol in utero. *Fertil Steril* 1995;63(6):1217–1221
18. Cohen J, Alikani M, Trowbridge J, Rosenwaks Z: Implantation enhancement by selective assisted hatching using zona drilling of human embryos with poor prognosis. *Hum Reprod* 1992;7: 685–691