Effects of Mullerian Anomalies on In Vitro Fertilization Outcome

KHALED I. ATTIA,¹ MARY HUG-KORONYA,¹ ELIZABETH S. GINSBURG,¹
and MARK D. HORNSTEIN^{1,2}

Submitted: October 11, 2000

Accepted: May 7, 2001

Purpose: To assess the effect of Mullerian anomalies on pregnancy rates in women undergoing in vitro fertilization (IVF).

Methods: The records of 37 patients with and 819 patients without Mullerian anomalies undergoing a first cycle of IVF between December 1995 and July 1998 were included in this retrospective study. Outcome variables included maximal estradiol level, number of days of stimulation, number of follicles, number of oocytes, fertilization rate, and ongoing/livebirth pregnancy rate.

Results: Patients with Mullerian anomalies had a significantly lower ongoing pregnancy rate (8.3%) than did controls (24.8%). No patients with diethylstilbestrol (DES)-related anomalies had an ongoing pregnancy.

Conclusions: Among women with Mullerian anomalies, those with DES exposure in utero demonstrated the poorest outcome.

KEY WORDS: DES; IVF; Mullerian anomalies; pregnancy rate.

INTRODUCTION

Advanced surgical techniques have improved the reproductive outcome for many women with uterine anomalies. Despite these surgical advances, many of these patients may ultimately require assisted reproductive treatments to conceive. In particular, in utero exposure to diethylstilbestrol (DES) has been associated with structural abnormalities of the female

reproductive tract and increased pregnancy loss. A direct effect on the ovaries, however, appears minimal (1). Although the effect of DES on reproduction in general is well known, its effect on in vitro fertilization (IVF) outcome has been examined in only a limited number of studies (2–5). In general, women exposed to DES fare poorly in IVF compared with women not exposed to DES. The role of non-DES Mullerian anomalies in IVF, however, has been less well studied than the role of DES-related Mullerian anomalies. In the only study specifically addressing the outcome of IVF in women with Mullerian anomalies, Marcus *et al.*, reported on a series of 24 patients with uterine congenital malformations (6) and found a 37% clinical pregnancy rate and a 25% live-birth rate per embryo transfer in their series. Patients with unicornuate uterus and uterus didelphys had the best outcomes. The purpose of our large retrospective study was to determine the effect of DES-related and non-DES-related Mullerian anomalies on IVF outcome.

MATERIALS AND METHODS

A total of 2,222 cycles performed at Brigham and Women's Hospital from December 1995 to July 1998 were reviewed. To prevent introduction of bias, we included only first cycles ($N = 998$) in this study. Of these patients, 55 had Mullerian anomalies.

The control group consisted of 943 patients without documented uterine anomalies. Patients with other potentially acquired uterine anomalies such as cervical stenosis, submucous myomas, endometrial polyps, intrauterine adhesions, and other intracavitary filling defects were excluded from either group. Eighteen patients and 124 controls were excluded because of incomplete data, leaving 37 patients with anomalies [DES ($n = 22$), uterine septum ($n = 12$), bicornuate

¹Department of Obstetrics and Gynecology, Brigham and Women's Hospital, Harvard Medical School, Boston, Massachusetts.

²To whom correspondence should be addressed at Department of Obstetrics and Gynecology, Brigham and Women's Hospital, 75 Francis Street, Boston, Massachusetts 02115; e-mail: mhornstein@partners.org.

uterus ($n = 2$), and uterus didelphys ($n = 1$)] and 819 controls.

All patients with anomalies had their abnormalities confirmed by hysterosalpingogram, sonohystero-gram, hysteroscopy, or laparoscopy. All patients with a uterine septum underwent surgical correction of the defect before IVF.

Controlled ovarian hyperstimulation generally was performed with luteal downregulation, using le-uprolide acetate (Lupron, TAP Pharmaceuticals, Deerfield, IL). After downregulation, stimulation was initiated with purified FSH (Fertinex, Serono Laboratories, Norwell, MA) or recombinant FSH (Follistim, Organon, West Orange, NJ; or Gonal-F, Serono Laboratories), using divided doses according to patient response. Ovulation was triggered with human chorionic gonadotropin (Profasi, Serono Laboratories) when lead follicles reached a maximal diameter of 20 mm and the level of estradiol exceeded 500 pg/mL. Transvaginal ultrasound-guided oocyte retrieval was performed 36 h later with embryo transfer 2, 3, or 5 days after retrieval. The luteal phase was supported with progesterone either as an intramuscular 50-mg injection or vaginal suppositories, generally 100 mg twice daily or 90 mg, 8% gel (Crinone, Serono Laboratories).

Pregnancies were defined as an increase in rising serum hCG level with ultrasound confirmation of an intrauterine gestation with normal fetal heart activity.

The study was approved as a medical records study by the Partners Healthcare System Human Research Committee. Interval data were compared using a two-sided Student's *t* test, and proportional data were compared using Fisher's exact test as appropriate. Data are reported as mean $\pm$ standard deviation. Statistical significance was assumed for $p < 0.05$.

RESULTS

There was no difference between the groups in age, levels of estradiol on the day of hCG, number of days of stimulation, number of follicles on the day of hCG, number of oocytes retrieved, or the fertilization rate (Table I). The ongoing pregnancy rate was significantly lower in women with Mullerian anomalies (8.3%) than in controls (24.8%), $P = 0.0178$ (Table I). Among patients with uterine anomalies, there was no significant difference between the pregnancy rate between those with non-DES malformations (20.0%) and the rate in those with DES-related anomalies (0%), $P = \text{NS}$. In addition, there was no difference between the pregnancy rate in patients with non-

Table I. Results in 856 IVF Cycles in Women With Mullerian Anomalies and Controls

	Mullerian anomalies ($N = 37$)	Controls ($N = 819$)	<i>P</i>
Age (years)	37.2 ± 4.1	35.1 ± 4.3	NS
Estradiol on day of hCG (pg/mL)	1778 ± 867	1852 ± 1034	NS
Days of stimulation	11.4 ± 1.6	11.3 ± 1.9	NS
Number of follicles ≥ 12 mm on day of hCG	12.2 ± 5.85	13.1 ± 7.3	NS
Number of oocytes retrieved	14.8 ± 9.7	15.6 ± 9.6	NS
Fertilization rate	57%	54%	NS
Ongoing pregnancy/ livebirth rate	8.3%	24.8%	0.0178

Note. NS – not significant.

DES malformations (20.0%) and the rate in controls (24.8%), $P = \text{NS}$.

Because the majority of the Mullerian anomalies were DES-related, this subgroup was also analyzed separately. The DES-exposed patients were older (38.2 ± 3.7 years) as compared with the controls (35.1 ± 4.3 years), $P = 0.001$ (Table II). There was no difference between the groups in levels of estradiol on the day of hCG, number of days of stimulation, number of follicles on the day of hCG, or number of oocytes retrieved. The fertilization rate was higher in DES-diagnosed patients (62%) than controls (54%), $P = 0.012$. The pregnancy rate in the DES group (0%) was significantly lower than that in controls (24.8%), $P = 0.0039$.

DISCUSSION

In this study, we found a lower pregnancy rate in IVF in women with uterine malformations than in

Table II. Results in 841 IVF Cycles in Women With In Utero DES Exposure and Controls

	DES-exposed ($N = 22$)	Controls ($N = 819$)	<i>P</i>
Age (years)	38.2 ± 3.7	35.1 ± 4.3	0.001
Estradiol on day of hCG (pg/mL)	1881 ± 999	1852 ± 1034	NS
Days of stimulation	11.1 ± 1.5	11.3 ± 1.9	NS
Number of follicles ≥ 12 mm	13.5 ± 1.5	13.1 ± 7.3	NS
Number of oocytes retrieved	16.3 ± 11.9	15.6 ± 9.6	NS
Fertilization rate	62%	54%	0.012
Ongoing pregnancy/ livebirth rate	0%	24.8%	0.0039

Note. NS – not significant.

women without uterine abnormalities. Furthermore, among patients with uterine malformations, the DES-exposed women had the poorest outcome, with no ongoing pregnancies in 22 cycles.

Intrauterine exposure to DES has long been known to be a factor in infertility. Although the mechanism is not yet understood, it has been suggested that DES affects the reproductive tract at multiple levels. In IVF cycles, the potential effects of DES may be most pronounced on the ovaries or the uterus.

Only a few studies have focused on the ovarian factor in infertile women exposed to DES in utero. Probably the best designed study on the effect of DES on the ovaries was provided by Sangvai *et al.* (1). They compared women with DES exposure in utero to a control group without DES exposure and evaluated the ovarian reserve as measured by the clomiphene citrate challenge test and response to ovarian stimulation by gonadotropins. Both the ovarian reserve and the response to stimulation were similar in terms of the total dose of human menopausal gonadotropins, the day of human chorionic gonadotropin administration, the peak estradiol level, and the number of mature follicles. The results of Sangvai *et al.* suggested that neither the ovarian reserve nor ovarian stimulation is affected in women exposed to DES. Kerjean *et al.* (2) noted similar results in an IVF program, which demonstrated no difference between DES-exposed women and women in the control group with regard to oocyte quality, fertilization rate, and number and quality of transferred embryos. The results of these studies strongly suggest that the hypothalamic-pituitary-ovarian axis is not affected in infertile women exposed to DES. In a small series of 21 cycles, Muasher *et al.* (4) also reported similar estradiol response and oocyte quality in DES-exposed patients compared with tubal factor patients. The pregnancy rate was 23.5% per cycle.

Contrary to the ovarian factor, the uterine factor may be affected by DES, as suggested by Noyes *et al.* (5), who compared the endometrial pattern and IVF outcome in women exposed to DES in utero with that in non-DES-exposed women. They found a higher frequency of a solid endometrial pattern in women with DES exposure and a statistically significant lower pregnancy rate in that group than in controls. Although the overall embryo implantation rate, pregnancy rate, and the delivery rate were not statistically different between DES- and non-DES-exposed women in the Noyes study, Karande *et al.* (7) noted a significantly worse outcome in ongoing pregnancy rates in patients with DES exposure than in women

with tubal factor infertility despite similar number of oocytes retrieved and embryos transferred. These studies suggest that the major factor limiting success in IVF in DES-exposed women is the uterine factor.

In the only other study to examine the relationship between congenital uterine malformations and IVF outcome, Marcus *et al.* (6) concluded that patients with Mullerian anomalies had a high success rate, with a clinical pregnancy rate of 37% per embryo transfer. There was, however, a high rate of preterm delivery (46%) and deliveries by cesarean section (77%). This reported series was small, consisting of only 24 patients, and included no control group.

This study focused on both women with DES-related and women with non-DES-related Mullerian anomalies undergoing IVF and compared them with a control group consisting of patients with no known uterine malformations. This study, unlike previous studies, examined IVF outcome in patients with DES- and non-DES-related uterine anomalies separately and compared them with a normal control group. Both the group with Mullerian anomalies and the control group showed normal ovulation and fertilization parameters. There was no difference between the groups in maximal estradiol, number of days of gonadotropin stimulation, average number of follicles on the day of hCG, number of oocytes retrieved, and the fertilization rate suggesting that ovarian function was unaffected in the subjects with Mullerian anomalies. The ongoing pregnancy rate for women with Mullerian anomalies (DES and non-DES) was 8.3% compared with 24.8% in the control group ($P = 0.0178$). Significantly, among the women with Mullerian anomalies, none of the 22 DES-exposed women maintained a pregnancy, whereas 3 of 12 women with a corrected uterine septum maintained a pregnancy (25.0%). It should be noted that the DES-exposed women were significantly older than controls, however the small difference in age (38.2 ± 4.3 years vs. 35.1 ± 4.3 years) alone cannot account for a 0% pregnancy rate in the DES-exposed patients.

Our findings and those of previous studies demonstrate normal ovarian function in women with Mullerian defects, but the lower pregnancy rate in these women suggests that a uterine factor is likely responsible for the significantly poorer outcome, especially in those women exposed to DES in utero.

In conclusion, patients with Mullerian anomalies, specifically those with DES-related malformations, appear to have a poor prognosis in IVF. Physicians

should use this information in counseling such patients about reproductive choices.

REFERENCES

1. Sangvai M, Thie J, Hofmann GE: The effect of intrauterine diethylstilbestrol exposure on ovarian reserve screening. *Am J Obstet Gynecol* 1997;177:568–572
2. Kerjean A, Poirot C, Epelboin S, Jouannet P: Effect of in-utero diethylstilboestrol exposure on human oocyte quality and fertilization in a programme of in-vitro fertilization. *Hum Reprod* 1999;14:1578–1581
3. Pal L, Shifren JL, Isaacson KB, Chang Y, Marean M, Leykin L, Toth TL: Outcome of IVF in DES-exposed daughters: Experience in the 90s. *J Assist Reprod Genet* 1997;14:513–517
4. Muasher SJ, Garcia JE, Jones HWJ: Experience with diethylstilbestrol-exposed infertile women in a program of in vitro fertilization. *Fertil Steril* 1984;42:20–24
5. Noyes N, Liu HC, Sultan K, Rosenwaks Z: Endometrial pattern in diethylstilboestrol-exposed women undergoing in-vitro fertilization may be the most significant predictor of pregnancy outcome. *Hum Reprod* 1996;11:2719–2723
6. Marcus S, Al-Shawat T, Brindsen P: The obstetric outcome of in vitro fertilization and embryo transfer in women with congenital uterine malformations. *Am J Obstet Gynecol* 1996;175:85–89
7. Karande VC, Lester RG, Muasher SJ, Jones DL, Acosta AA, Jones HW, Jr: Are implantation and pregnancy outcome impaired in diethylstilbestrol-exposed women after in vitro fertilization and embryo transfer? *Fertil Steril* 1990;54:287–291