

**Case report****Estradiol and progesterone supplementation during luteal phase improved the receptivity of the endometrium in a patient with a history of diethylstilboestrol exposure in-utero**

Dimitris Loutradis,<sup>1</sup> Konstantinos Stefanidis,<sup>1</sup> Erasmia Kiapekou,<sup>2</sup> Evangelia Zapanti,<sup>2</sup> Chrisoula Panitsa-Fafli,<sup>1</sup> Aristidis Antsaklis<sup>1</sup>

<sup>1</sup>First Department of Obstetrics and Gynaecology, Athens University School of Medicine, Alexandra Hospital,

<sup>2</sup>First Endocrine Section, Alexandra Hospital, Athens, Greece

**ABSTRACT**

**BACKGROUND:** Diethylstilboestrol (DES) exposure in-utero has been shown to have negative effects on pregnancy. DES-exposed women are at increased risk of early spontaneous pregnancy loss, ectopic gestation and infertility. **DESIGN:** A 34-year old woman with a 6-year history of primary infertility is presented. The patient underwent in vitro fertilization (IVF) treatment without success. To improve the quality of the endometrium following IVF treatment, E2 and progesterone supplementation was added to the usual therapeutic regimen. The pregnancy progressed uneventfully and a normal female was born. **CONCLUSIONS:** This case indicates that the administration of E2 and progesterone in DES-exposed women might improve endometrium receptivity and consequently pregnancy outcome.

**Key words:** DES, Estradiol, IVF, Progesterone, Implantation

**INTRODUCTION**

Diethylstilboestrol (DES) exposure in-utero has been shown to have a potentially negative impact on pregnancy. Negative effects include an increased risk of early pregnancy loss,<sup>1,2</sup> ectopic gestation<sup>1</sup> and infertility.<sup>3-5</sup> These women may also present reproductive tract abnormalities leading to pregnancy complications. The most common anomalies include uterine defects such as T-shaped uterus or hypo-

plastic uterine cavity.<sup>6</sup>

From the late 1940s until 1971 an estimated 2 to 3 million women were prescribed DES during their pregnancies; thus, approximately 3 million embryos were exposed to the drug in-utero.<sup>6</sup>

Karande et al. (1990)<sup>7</sup> and Noyes et al (1996)<sup>8</sup> presented data suggesting that among women undergoing *in-vitro* fertilization (IVF), in-utero DES-exposed infertile women experienced decreased success when compared with patients with other infertility factors. The authors suggested that the endometrial pattern as well as "uterine receptivity" are important factors for pregnancy success and should

Address correspondence and requests for reprints to:  
Kiapekou Erasmia, Karaiskaki 25, 157 72 Athens, Greece,  
Tel.: +30-210-7488926, E-mail: nem10@panafonet.gr

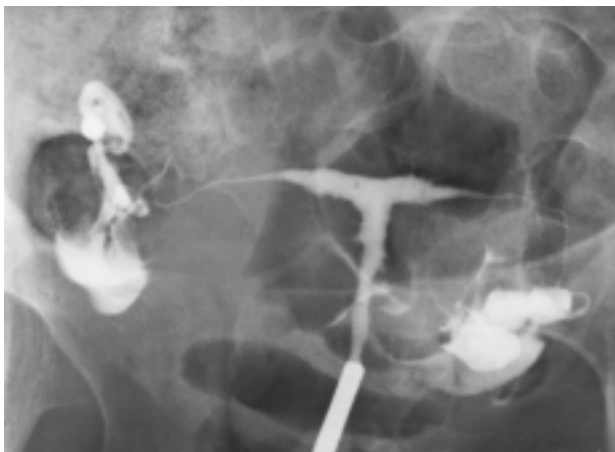
Received 19-12-05, Revised 28-02-06, Accepted 10-03-06

be seriously taken into account.

In the present report we describe an in-utero DES exposed woman enrolled in an IVF program. The protocol that was employed for this case was the protocol used for donor patients in order to improve the receptivity of the endometrium.<sup>9</sup>

## CASE REPORT

A 34-year old woman presented with a 6-year history of primary infertility. Her menstrual cycle was regular (every 26-33 days). She had a history of DES exposure in-utero. A T-shaped uterus and normal ovaries were found by hysterosalpingography (HSG) (Figure 1). Routine biochemical data and pertinent hormonal results were normal. The patient underwent 6 cycles of intra-uterus insemination (IUI) without success and subsequently was enrolled in an IVF program. Firstly the long GnRH protocol was used as follows: On day 21 of the previous cycle, a baseline ultrasound scan was performed. Simultaneously, a GnRH agonist nasal spray was commenced (buserelin, Hoechst, Germany) at a dose of 100 µg 5 times daily (every 4h, omitting the 3 a.m. dose). On this long protocol, the down-regulation period before FSH administration was 15-24 days. The extent of ovarian suppression was evaluated by ultrasound scan and serum E2 levels (= 40 pg/ml). Subsequently, stimulation was initiated with human recombinant FSH (Puregon, Or-



**Figure 1.** Utero-salpingography showing T-shape uterine cavity, typical of the DES syndrome.

ganon, the Netherlands) at a fixed dose of 200 IU daily. GnRH-agonist administration was continued until human chorionic gonadotropin (hCG) was administered (Pregnyl, Organon, the Netherlands). The latter was given intramuscularly at a dose of 10,000 IU when the mean diameter of at least two leading follicles was > 18 mm and serum E2 was rising. 35-36h after hCG administration, ovum retrieval was performed by transvaginal echo-guided ovarian puncture. The luteal phase was supported with 2500 IU hCG injected on days of ovum retrieval, embryo transfer and day 4 after embryo transfer. Seven oocytes were collected, 5 of which were fertilized. Estradiol levels on the day of HCG administration were 1867 pg/ml, while the endometrial pattern on the day of retrieval was "solid" according to Noyes criteria.<sup>8</sup> The embryo transfer (ET) was performed on day 3 after hCG administration. The embryos transferred were at the 8-cell stage and their quality was "excellent". The subsequent β-hCG test was negative for pregnancy.

The next cycle was repeated after a 5-month interval. The same protocol for ovulation induction was used. Estradiol levels on the day of hCG administration were 2100 pg/ml and 8 oocytes were retrieved. Unfortunately, the pattern of the endometrium was "solid" again. Thus, the administration of 6 mg/day estradiol per-os and 200µg/week transdermally was decided upon from the day of ovum pickup. The day before embryo transfer, progesterone supplementation at 800mg/day divided between 300mg/day per os and 500mg/day vaginally was given. On day 3, three embryos of good quality at 8-cell stage were transferred. The pregnancy test was positive 15 days after ET and ultrasound investigation revealed an intact, intrauterine, singleton pregnancy. The administration of E2 and progesterone were continued until the 28<sup>th</sup> week of gestation. A normal female baby was delivered at the 37<sup>th</sup> gestational week by C. Section with a birth weight of 2400gr.

## DISCUSSION

The present case report suggests that the administration of E2 and progesterone had a beneficial effect on the receptivity of the endometrium in this

infertile woman who was DES- exposed in-utero and who had experienced a failure to conceive on one previous attempt of IVF. In both attempts the failure was attributed to the “solid” pattern of the endometrium on the day of retrieval.

Noyes et al.<sup>8</sup> compared the pregnancy outcome, as it is related to ultrasound endometrial pattern, in in-utero DES- and non-DES-exposed women and suggested that the ultrasound endometrial pattern is an important variable for pregnancy outcome in in-utero DES-exposed women undergoing IVF. According to Noyes *et al.*, endometrial patterns were assigned as p1= solid, p2= ring, p3= intermediate. In-utero DES-exposed patients showed a solid endometrium pattern more often than in-utero non-DES-exposed women. The same authors reported that no pregnancies resulted after IVF in in-utero DES-exposed patients with “solid” configuration of the endometrium as compared with 36.5% delivery rate in in-utero non-DES-exposed controls. In cycles in which the endometrium pattern of the women was “ring” and “intermediate”, there was no significant difference between DES exposed in-utero and non-exposed individuals with regard to pregnancy rates. Thus, it seems that the endometrium pattern is of importance for pregnancy outcome in DES in-utero exposed women undergoing IVF.

Salle et al.<sup>10</sup> demonstrated that the endometrial thickness was decreased significantly in in-utero DES-exposed women compared to the non-exposed healthy controls, particularly in the luteal phase. This may reflect insufficient endometrial support for the early conceptus leading to infertility or early spontaneous abortion. This study also documented that the pulsatility index of the uterine arteries in the in-utero DES-exposed group was higher throughout the cycle and failed to undergo a decrease in the luteal phase.

Serum values of E2 and progesterone decline from the midluteal phase in IVF-ET cycles in which pituitary suppression is used for controlled ovarian hyperstimulation. This decline has been found to adversely affect implantation, particularly in patients who are treated with the long GnRH analog protocol for controlled ovarian hyperstimulation.<sup>11-13</sup> The addition of E2 and progesterone to this regimen had

a beneficial effect on pregnancy and implantation rates.<sup>14</sup> The term “uterine receptivity” was introduced first in the animal models and subsequently in humans to denote a window of implantation beyond which egg transfer is unsuccessful and could be a major factor limiting IVF pregnancy rates.<sup>15</sup>

Although progesterone secretion is considered the main hormonal event during the luteal phase, E2 appears to play a crucial role as well. The pinopodes developing on the apical surface of the luminal uterine epithelium appearing in normal cycles at the peri-implantation period are now used as a morphological marker of endometrium receptivity. The effect of the duration of E2 administration on pregnancy outcome has been evaluated clinically in the study of Nikas et al.<sup>16</sup> The authors found that the effectiveness of the regimen was strongly correlated with the duration of the artificial follicular phase, with the best results achieved when the E2 was administered from 6 to 11 days. Progesterone administration was not found to affect the outcome.<sup>9</sup> Furthermore, it has been demonstrated that in patients who were treated with the long GnRH-agonist protocol, the addition of E2 and progesterone support regimen had a beneficial effect on pregnancy and implantation rate.<sup>11</sup>

The use of the protocol described in the present case aimed at targetting the window of implantation, through enhancement of the endometrial receptivity, as well as the improvement of the configuration of “solid” pattern endometrium. The same protocol has also been used in patients enrolled in an oocyte donation program with achievement of a high success rate. In these cases the endometrial receptivity was induced successfully in recipients by employing a constant administration of estradiol valerate and progesterone.<sup>9,16</sup>

An explanation for the failure of implantation in in-utero DES-exposed patients was advanced by Bern *et al.* in 1987.<sup>17</sup> Their findings lend support to a Mullerian rather than an endocrine etiology. Moreover, Miller et al.<sup>18</sup> and Ma et al.<sup>19</sup> presented evidence that antenatal DES exposure disrupts expression of *Wnt7a*, *Hoxa10* and *Hoxa11* in the upper Mullerian duct, while other authors in the past had identified various genes as potential candidates

of DES-induced abnormalities in the female reproductive tract.

The present case report suggests the potential of improvement of endometrial receptivity in in-utero DES-exposed women by estradiol and progesterone supplementation. Further studies are needed to confirm its effectiveness and define the requirements of E2 and progesterone supplementation for improvement of the pregnancy rates in these women.

Although the favorable outcome in our patient may represent a chance association we consider the case worth reporting. The accumulation of more data will verify or disprove the effectiveness of the proposed therapeutic regimen in in-utero DES exposed women.

## REFERENCES

- Herbst AL, Senekjian EK, Frey KW, 1989 Abortion and pregnancy loss among diethylstilbestrol-exposed women. *Semin Reprod Endocrinol* 7: 124-129.
- Levine RU, Berkowitz KM, 1993 Conservative management and pregnancy outcome in diethylstilbestrol-exposed women with and without gross genital tract abnormalities. *Am J Obstet Gynecol* 169: 1125-1129.
- Herbst AL, Hubby MM, Azizi F, et al, 1981 Reproductive and gynecologic surgical experience in diethylstilbestrol-exposed daughters. *Am J Obstet Gynecol* 141: 1019-1028.
- Mangan CE, Borow L, Burnett-Rubin MM, et al, 1982 Pregnancy outcome in 98 women exposed to diethylstilbestrol in utero, their mothers, and unexposed siblings. *Obstet Gynecol* 59: 315-319.
- Senekjian EK, Potkul RK, Frey K, et al, 1988 Infertility among daughters either exposed or not exposed to diethylstilbestrol. *Am J Obstet Gynecol* 158: 493-498.
- Kaufman RH, Binder GL, Gray PM, Adam E, 1977 Upper genital tract changes associated with exposure in utero to diethylstilbestrol. *Am J Obstet Gynecol*, 128: 51-54.
- Karande VC, Lester RG, Muasher JS, Jones DL, Acosta AA, Jones HW, 1990 Are implantation and pregnancy impaired in diethylstilbestrol-exposed women after in vitro fertilization and embryo transfer? *Fertil Steril* 54: 287-291.
- Noyes N, Liu CH, Sultan K, Rosenwa SSZ, 1996 Endometrial pattern in diethylstilbestrol-exposed women undergoing in vitro fertilization may be the most significant predictor of pregnancy outcome. *Hum Reprod* VII, No 12: 2719-2723.
- Michalakis S, Loutradis D, Drakakis P, et al, 1996 A flexible protocol for the induction of recipient endometrial cycles in an oocyte donation programme. *Hum Reprod* 11: 1063-1066.
- Salle B, Sergeant P, Awada A, et al, 1996 Transvaginal ultrasound studies of vascular and morphological changes in uteri exposed to diethylstilbestrol in utero. *Hum Reprod* 11: 2531-2536.
- Huchinson-Williams K, Decherney A, Lavy G, Diamond MP, Naftolin F, Lunenfeld B, 1990 Luteal rescue in in vitro fertilization-embryo transfer. *Fertil Steril* 3: 495-501.
- Smith E, Anthony F, Gadd S, Masson G, 1989 Trial of support treatment with human chorionic gonadotropin in the luteal phase after treatment with buserelin and human gonadotropin in women taking part in an in vitro fertilization program. *Br Med J* 298: 1483-1486.
- Herman A, Ron-El R, Golan A, Raziel A, Soffer Y, Caspi E, 1990 Pregnancy rate and ovarian hyperstimulation after luteal human chorionic gonadotropin in in vitro fertilization stimulated with gonadotropin-releasing hormone analog and menotropins. *Fertil Steril* 1: 92-96.
- Farhi J, Weissman A, Steinfeld Z, Shorer M, Nahum H, Levran D, 2000 Estradiol supplementation during the luteal phase may improve the pregnancy rate in patients undergoing in vitro fertilization-embryo transfer cycles. *Fertil Steril* 73: 761-766.
- Psychoyos A, Martel D 1985 Embryo-endometrial interactions at implantation. In: Edwards RG, Purdy JM, Steptoe PC (eds) *Implantation of the Human Embryo*, Academic Press, London, UK; p, 195.
- Nikas G, Drakakis P, Loutradis D, et al, 1995 Uterine pinopodes as markers of the "nidation window" in cycling women receiving exogenous oestradiol and progesterone. *Hum Reprod* 10: 1208-1213.
- Bern HA, Edery M, Mills KT, et al, 1987 Long term alterations in histology and steroid receptor levels of the genital tract and mammary gland following neonatal exposure of female BALB/cCrJ mice to various doses of diethylbestrol. *Cancer Res* 47: 4165-4172.
- Miller C, Degenhardt K, Sassoon DA, 1998 Fetal exposure to DES results in de-regulation of Wnt7a during uterine morphogenesis. *Nat Genet* 20: 228-230.
- Ma L, Benson GV, Lim H, Dey SK, Maas RL, 1998 Abdominal B (AbdB) Hoxa genes: regulation in adult uterus by estrogen and progesterone and repression in müllerian duct by the synthetic estrogen diethylstilbestrol (DES). *Dev Biol* 197: 141-154.