CLINICAL ASSISTED REPRODUCTION

Subcutaneous Estradiol Pellets for Endometrial Preparation in Donor Oocyte Recipients with a Poor Endometrial Response¹

W. PAUL DMOWSKI,^{2,3} JANINA MICHALOWSKA,² NASIRUDDIN RANA,² JAN FRIBERG,² ELIZABETH MCGILL-JOHNSON,² and LINDA DeORIO²

Submitted: September 18, 1996

Accepted: October 21, 1996

Purpose: The purpose of this study was to evaluate the clinical effectiveness of subcutaneous estradiol pellets in donor oocyte recipients with an inadequate endometrial response.

Methods: The subjects were 13 women with ovarian failure and a maximal endometrial thickness <10 mm on standard estrogen regimens, as demonstrated during mock and/or prior oocyte donation cycles. They underwent pellet implantation (100–250 mg of estradiol) 6–13 weeks before oocyte donation.

Results: Maximal (mean $\pm$ SD) endometrial thickness was 8.7 ± 1.5 mm on standard regimens, in contrast to 11.7 ± 1.8 mm on pellets, while estradiol levels were 674 ± 844 and 815 ± 706 pg/ml, respectively. The estradiol:estrone ratio on pellets was >1 . There was 1 pregnancy with early loss during 10 cycles on other estrogen regimens and 12 pregnancies during 19 cycles on pellets. The pregnancy and implantation rates were, respectively, 63 and 27% on pellets and 41 and 14% on standard regimens in historical controls.

Conclusions: We conclude that estradiol pellets after a single administration provide constant estradiol levels extending into the first trimester of pregnancy, a physiologic estradiol:estrone ratio, and a better endometrial response than standard estrogen regimens. Implantation and preg-

nancy rates are higher. This approach may be especially suitable for recipients with a poor endometrial response.

KEY WORDS: estradiol pellets; donor oocyte recipients; endometrial thickness; implantation rates; pregnancy rates; oocyte donation.

INTRODUCTION

The first human pregnancy achieved through in vitro fertilization (IVF) from a donated oocyte was reported in 1983 by Trounson *et al.* (1). The oocyte donor was a 42-year-old infertile woman and the pregnancy terminated in a spontaneous first-trimester abortion because of fetal trisomy. During the following decade, oocyte donation became a highly effective method for the treatment of infertility secondary to ovarian failure (2–7). There is no clear understanding as to the mechanism of high fecundity with donated oocytes, and various explanations have been proposed (8,9). It is generally agreed that there is a natural age-related decline in the human fertility and that oocytes from younger women should have a higher potential for establishing pregnancy. There is also evidence of age-related changes in the uterine receptivity (8). It has been suggested that uterine receptivity is related to the endometrial response to estrogenic stimulation, as indicated by the endometrial thickness, and that a decrease in the endometrial thickness is associated with lower pregnancy rates in recipients of donated oocytes (10).

A variety of estrogenic preparations has been recommended to stimulate endometrial proliferation and to

¹ Presented at the IXth World Congress on In Vitro Fertilization and Assisted Reproduction, Vienna, Austria, April 3, 1995, and the 51st Annual Meeting of the American Society for Reproductive Medicine, Seattle, Washington, October 7–12, 1995.

² Family Fertility Center, Chicago, and Oak Brook Fertility Center, Oak Brook, Illinois.

³ To whom correspondence should be addressed at Columbia Grant Hospital, 550 West Webster Avenue, Chicago, Illinois 60614.

increase endometrial thickness in donor oocyte recipients without endogenous ovarian function. Oral estradiol valerate (11), oral estradiol with estriol at a 2:1 ratio (12), oral micronized estradiol (3), transdermal estradiol patches (6), estradiol in a silastic vaginal ring (3), micronized estradiol vaginal tablets (13), and intramuscularly (im) injectable estradiol valerate (14) have all been used with varying degrees of success. Each one of these methods requires repeated, frequent administrations, and all have individually variable absorption rates and variable bioavailability and give variable serum estrogen levels. Oral preparations have an undesirable first-pass effect on the liver, and all, with the exception of the parenteral estradiol valerate, provide an unphysiologic serum estradiol:estrone ratio. Feinman *et al.* (14) reported recently that im estradiol valerate may in many respects be superior to oral or transdermal estrogens, but even this preparation requires repeated injections and provides widely fluctuating serum estradiol levels.

Estradiol pellets implanted subcutaneously (sc) have been used for many years as a form of estrogen replacement therapy. After a single sc implantation, they release estradiol into the peripheral circulation at a steady state for approximately 6 to 10 months (15). With such a form of estradiol administration, there is no first-pass effect on the liver, the estradiol:estrone ratio is >1 , and there is no need for repeated administrations. Estradiol pellets may, therefore, be a preferable estrogen replacement therapy in recipients of donated oocytes.

For the purpose of this study, we evaluated, in donor oocyte recipients with a poor endometrial response, the effect of sc estradiol pellets on the endometrial thickness during mock and embryo transfer cycles, on serum estradiol levels before and after embryo transfer, and on the outcome of the oocyte donation cycles.

MATERIALS AND METHODS

Subjects. The subjects in this study were 13 women, age 27–49, with ovarian failure, requesting IVF as recipients of donated oocytes. Three had known egg donors and 10 were matched with egg donors unknown to them. Ten of 13 underwent prior IVF/egg donation as recipients, using standard estrogen replacement regimens; 9 failed to conceive, and 1 conceived but had early pregnancy loss. The age and clinical characteristics of all recipients are listed in Table I. All 13 had a maximal endometrial thickness on standard estrogen replacement regimens of <10 mm, and all were diag-

nosed as having inadequate endometrial growth. In order to improve the endometrial response the subjects were offered sc estradiol pellets. On pellets, 13 recipients underwent 10 mock and 19 IVF/egg donation cycles. To assess the response to estrogen replacement, the subjects were evaluated three times a week during mock cycles and prior to embryo transfer and at weekly intervals during ensuing pregnancies. Serum estradiol and endometrial thickness were measured at each visit during mock and embryo transfer cycles, and serum estradiol during the first trimester of pregnancies. Serum estrone was measured in two patients on estradiol pellets during two mock cycles. The thickness of both layers of the endometrium, the so-called endometrial stripe, was measured sonographically and recorded as the mean of two maximal diameters obtained in sagittal and transverse sections. Maximal endometrial thickness for a particular patient was considered when (a) estrogen replacement was continued for at least 10 days and the progressively increasing endometrial thickness reached ≥ 10 mm, which was considered physiological, or (b) when estrogen replacement was continued for at least 20 days, serum estradiol levels were above 500 pg/ml, but endometrial thickness was <10 mm, and there was no endometrial growth during three successive visits, which was considered inadequate. For the purpose of comparison, 23 cycles on pellets and 10 on other regimens were available in 13 recipients. The maximal endometrial thickness the day before embryo transfer was available for comparison in the same seven recipients on estradiol pellets and on other estradiol regimens. To evaluate the outcome of IVF/egg donation cycles on estradiol pellets, we analyzed oocyte donation cycle parameters including pregnancy rates on estradiol implants (19 cycles), and on other estradiol regimens (10 cycles) in the same recipients, and compared them to those in 49 consecutive oocyte donation cycles performed in our program using other estrogen regimens (historical controls). The endometrial thickness and estradiol levels in historical controls were measured infrequently during egg donation cycles and could not be used for comparison with the study group.

Estradiol pellets, 25 mg each, were prepared by College Pharmacy (Colorado Springs, CO) and supplied in sterile vials. A total dose of 100–250 mg of estradiol (4–10 pellets) was implanted sc into the adipose tissue of the anterior abdominal wall, 6 to 13 weeks before oocyte donation. The pellets were implanted after local infiltration of the skin with 1–2 ml of xylocaine using aseptic technique and Kearn's pellet implanter. They dissolved completely and did

Table I. Clinical Data and the Outcome of Oocyte Donation Cycles in Recipients who Received sc Estradiol Pellets^a

No.	Initials	Age	Parity	Diagnosis	Max ET (mm)		Donor ^b	Pellet dose (mg)	No. cycles	Outcome
					Other regimens	Pellets				
1	AD	49	0000	Ovarian failure	9	12	K	250	2	1 miscarriage
2	KJ	49	0000	Ovarian failure	9	13	U	250	1	♀ ♀ ♂
3	BL	44	0000	Ovarian failure	8	11	U	250	2	1 miscarriage 1 ♂
4	MJ	40	1001	Ovarian failure	9	10	U	250	2	♀
5	SK	32	0000	Ovarian dysgenesis, 45X/46XX	8	13	U	250	2	1 ♀ 1 ongoing
6	AM	29	0000	Ovarian dysgenesis, 45X/46XX	8	12	U	250	3	1 ♀ 1 ongoing
7	KK	36	0010	S/P ovarian radiation	9	10	U	200	1	♀
8	JL	29	0000	Ovarian dysgenesis	8	13	U	250	1	♀ ♀
9	GJ	36	0000	S/P oophorectomy	8	11	U	100	1	Not pregnant
10	ZS	43	2012	Ovarian failure	8	10	K	250	1	♀
11	GD	43	0000	Ovarian failure	7	10	K	250	1	Not pregnant
12	TP	43	0000	Ovarian failure	8	8	U	250	1	Not pregnant
13	TL	27	0000	Ovarian failure	8	8	U	250	1	Not pregnant

^a Max ET, maximal endometrial thickness; S/P, status post; ♀, female infant delivered; ♂, male infant delivered.

^b K, known donor; U, unknown donor.

not require removal. Other estrogen regimens included micronized oral estradiol tablets at doses of 1–6 mg per day, estradiol transdermal systems at 0.2–0.4 mg per day, and estradiol valerate injections of 2–6 mg twice a week as recommended previously (6,11,13). Pelvic ultrasounds were performed using a real-time convex scanner with a 5.0-MHz transvaginal transducer (SAL-77B; Toshiba Medical Systems, Tustin, CA). Serum estradiol levels were measured using enzyme-linked immunosorbent assay (Enzymun-Test) and Boehringer Mannheim Automated Immunoassay Systems (Boehringer Mannheim Corp., Indianapolis, IN). Intraassay and interassay coefficients of variation were 6.4 and 12.5%, respectively. Serum estrone was measured using a double-antibody radioimmunoassay (RIA) system, supplied by Diagnostic Systems Laboratories, Inc., Webster, TX, with intraassay and interassay coefficients of variation of 7.2 and 14.5%, respectively. Statistical comparisons were performed using paired and unpaired Student's *t* tests, Mann–Whitney *U* test, and Kruskal–Wallis one way ANOVA, with *P* < 0.05 as the level of significance.

RESULTS

The maximal endometrial thickness prior to progesterone administration was significantly increased on estradiol pellets compared to other estrogen regimens,

as was the endometrial thickness during progesterone administration 1 day before embryo transfer (Tables I and II). There were also fewer days of estrogen stimulation required to achieve maximal endometrial thickness in spite of comparable serum estradiol levels. Mean ($\pm$ SD) serum estradiol levels after pellet implantation were 590 ± 382 pg/ml (conversion factor to SI units, 3.67) during donation cycles and the first 6–8 weeks of pregnancy and remained relatively constant during the period of study. The mean serum estrone level was 475 ± 169 pg/ml. Mean ($\pm$ SE) weekly estradiol levels during egg donation cycles and the first trimester of ensuing pregnancies are illustrated in Fig. 1 for patients who did and did not conceive and in Fig. 2 for continuing pregnancies and miscarriages. There was slight but not significant difference in mean endometrial thickness on pellets between patients who did and patients who did not conceive (11.04 ± 1.49 vs 10.52 ± 1.10 mm, respectively). The clinical data and the outcome of 19 oocyte donation cycles in 13 recipients are given in Table I. Twelve pregnancies occurred during 19 cycles of oocyte donation; 8 of these delivered at term, 2 miscarried, and 2 are ongoing. There was one set of triplets and one set of twins delivered. The analysis of IVF cycle parameters on estradiol pellets and on other regimens by comparison to historical controls is given in Table III. There was one conception and early pregnancy failure during 10 cycles on other estrogen regimens, while on estradiol

Table II. Effect of Estradiol Pellets or Other Estrogen Regimens on Endometrial Thickness and Serum Estradiol Levels (Mean $\pm$ SD)

	Estradiol Pellets	Other regimens	P
Number of cycles	23	10	
Days to max ET ^a	13.1 $\pm$ 3.9	21.1 $\pm$ 4.9	<0.0001
Max ET (mm)	11.7 $\pm$ 1.8	8.7 $\pm$ 1.5	<0.0001
Serum estradiol at max ET (pg/ml)	814.7 $\pm$ 706.3	673.8 $\pm$ 843.5	NS
ET day before embryo transfer (mm) <i>n</i> = 7	12.4 $\pm$ 1.9	9.3 $\pm$ 1.1	<0.003 ^b

^a Max ET, maximal endometrial thickness.^b Paired *t* test.

pellets 12 conceptions occurred during 19 cycles of embryo transfer. Ten of these delivered or are ongoing, while two miscarried, for pregnancy, delivery/ongoing, and implantation rates of 63, 42, and 27%, respectively. In comparison, pregnancy, delivery, and implantation rates in historical controls were 41, 29, and 14%, respectively ($P < 0.01$).

DISCUSSION

We report here a new approach to estrogen replacement therapy in donor oocyte recipients undergoing

endometrial preparation for embryo transfer. The pellets are prepared from crystalline estradiol and are implanted sc. As indicated by previous studies (15), the release rate is relatively constant over a period of 6–12 months. This eliminates the need for repeated estrogen administration to a recipient who may already be taking other parenteral medications including progesterone. During pregnancy, estradiol pellets seem to have no adverse effects on placental estradiol production, as indicated in Figs. 1 and 2. Placental contribution to the estradiol pool seems to begin during the sixth to seventh week of gestation, as indicated by the incremental estradiol rise. The pellets release estradiol

Fig. 1. Serum estradiol levels (mean $\pm$ SE) after pellet implantation in patients who did ($n = 8$) and did not ($n = 4$) conceive during egg donation cycles.

Fig. 2. Serum estradiol levels (mean $\pm$ SE) after pellet implantation in patients who conceived during egg donation cycles and had either normal pregnancies ($n = 6$) or early miscarriages ($n = 2$).

directly into the peripheral circulation, thus avoiding the first pass through the liver and potentially undesirable effects. Furthermore, the circulating estradiol:estrone ratio is more physiological as demonstrated before (15) and confirmed in this study.

A constant 24-hr release into the peripheral circulation may be more beneficial than the fluctuating estradiol levels following repeated oral, transdermal, or im administrations. We have used doses ranging from 100

to 250 mg implanted sc. A 250-mg sc implant was consistently followed by mean estradiol levels ranging between 500 and 800 pg/ml, adequate in most patients for normal endometrial growth.

For the purpose of this study, we selected 13 donor oocyte recipients, 10 of whom had prior failure with donor oocyte IVF, and all had evidence of inadequate endometrial growth during embryo donation and mock cycles. As indicated in Tables I and II, the maximal endometrial thickness was higher on estradiol pellets than on other estrogen regimens, and the number of days to achieve maximal endometrial thickness was significantly lower. Considering that serum estradiol at the time of maximal endometrial thickness was not statistically different between pellets and other regimens, the data suggest that continuous estradiol release from the pellets may be more beneficial than the fluctuating levels resulting from repeated oral, transdermal, or im administrations.

The success in terms of implantation, pregnancy, and delivery rates in our 13 subjects during 19 cycles on pellets was much higher than during 10 cycles on other estrogen regimens. The subjects, however, were preselected by prior donor egg IVF failure, presumably secondary to prior poor endometrial response. Therefore, we compared IVF-egg donation cycles in our subjects to 49 consecutive oocyte donation cycles in recipients with normal endometrial response on stan-

Table III. Outcome of IVF/Egg Donation Cycles Using Estradiol Pellets or Other Estrogen Regimens for Stimulation of Endometrial Growth*

	Study subjects		Historical controls, other regimens
	Other regimens	Pellets	
No. of recipients	10	13	33
No. of transfers/cycles	10	19	49
No. of clinical pregnancies	1	12	20
% per transfer/cycle	10	63	41
No. of miscarriages (%)	1 (100)	2 (17)	6 (30)
No. of delivered/ongoing pregnancies	0	10	14
% per transfer/cycle	0	53	29
No. ongoing	0	2	0
Implantation rate (%)	—	27	14

* Differences between the groups in pregnancy, delivery, and implantation rates were significant at $P < 0.01$, Kruskal-Wallis one-way ANOVA.

dard estrogen regimens (historical controls). The implantation, pregnancy, and delivery rates on estradiol pellets were also significantly higher than on other regimens. Two of four patients on estradiol pellets who did not conceive had a persistently low endometrial thickness, which might have contributed to the conception failure (Table I). However, the difference in the maximal endometrial thickness between patients who did and patients who did not conceive was statistically not significant, presumably because of the small number of subjects.

In summary, sc estradiol pellets in donor oocyte recipients is a new approach to endometrial preparation which has several advantages and good patient acceptance. This approach may be of special value in patients for whom other estrogen regimens are unacceptable or ineffective.

REFERENCES

1. Trounson A, Leeton JF, Besanko M, Wood C, Conti A: Pregnancy established in an infertile patient after transfer of a donated embryo fertilized in vitro. *Br Med J* 1983;286:835-838
2. Leeton J, Trounson A, Wood C: The use of donor eggs and embryos in the management of human infertility. *Aust NZ J Obstet Gynaecol* 1984;24:265-270
3. Rosenwaks Z: Donor eggs: Their application in modern reproductive technologies. *Fertil Steril* 1987;47:895-909
4. Sauer MV, Paulson RJ, Macaso TM, Francis-Hernandez M, Lobo RA: Establishment of a nonanonymous donor oocyte program: preliminary experience at the University of Southern California. *Fertil Steril* 1989;52:433-436
5. Kennard EA, Collins RL, Blankstein J, Schover LR, Kanoti G, Reiss J, Quigley MM: A program for matched, anonymous oocyte donation. *Fertil Steril* 1989;51:655-660
6. Navot D, Bergh PA, Williams M, Garrisi GJ, Guzman I, Sandler B, Fox J, Schreiner-Engel P, Hofmann GE, Grunfeld L: An insight into early reproductive processes through the in vivo model of ovum donation. *J Clin Endocrinol Metab* 1991;72:408-414
7. Flamigni C, Borini A, Violini F, Bianchi L, Serrao L: Oocyte donation: Comparison between recipients from different age groups. *Hum Reprod* 1993;8:2088-2092
8. Edwards RG: Why are agonadot and post-amenorrhoeic women so fertile after oocyte donation? *Hum Reprod* 1992;7:733-734
9. Asch RH: High pregnancy rates after oocyte and embryo donation. *Hum Reprod* 1992;7:734
10. Abdalla HI, Brooks AA, Johnson MR, Kirkland A, Thomas A, Studd JWW: Endometrial thickness: A predictor of implantation in ovum recipients? *Hum Reprod* 1994;9:363-365
11. Lutjen P, Trounson A, Leeton J, Findlay J, Wood C, Renou P: The establishment and maintenance of pregnancy using in vitro fertilization and embryo donation in a patient with primary ovarian failure. *Nature* 1984;307:174-175
12. Navot D, Laufer N, Kopolovic J, Rabinowitz R, Birkenfeld A, Lewin A, Granat M, Margalioth EJ, Schenker JG: Artificially induced endometrial cycles and establishment of pregnancies in the absence of ovaries. *N Engl J Med* 1986;314:806-811
13. Shushan A, Simon A, Reubinoff BE, Lewin A, Schenker JG, Laufer N: The use of vaginal tablets as a vehicle for steroid replacement in a donor oocyte program. *Fertil Steril* 1994;62:412-414
14. Feinman M, Sher G, Massaranni G, Vaught L, Andreyko J, Salem R, Zouves C, Dodge S: High fecundity rates in donor oocyte recipients and in-vitro fertilization surrogates using parenteral oestradiol valerate. *Hum Reprod* 1993;8:1145-1147
15. Kapetanakis E, Dmowski WP, Auletta F, Scommegna A: Endocrine and clinical effects of estradiol and testosterone pellets used in long-term replacement therapy. *Int J Gynaecol Obstet* 1982;20:387-399