CLINICAL ASSISTED REPRODUCTION

Effect of Duration of Estradiol Replacement on the Outcome of Oocyte Donation

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Purpose: *To investigate if duration of estrogenic endometrial stimulation can affect recipient pregnancy rate in an ovum donation program.*

Methods: *Each recipient received micronized 17β-estradiol orally in a steadily increasing dosage from 2 to 6 mg daily over a period of time varying from 5 to 76 days until oocyte were available for donation. Recipients (520 patients for a total of 835 transfer cycles) were retrospectively divided into five groups depending on the duration of E_2 administration.*

Results: *No significant difference was seen in pregnancy and implantation rates between groups. There was a higher number of miscarriages in Group A (41%), $p < 0.05$ vs. Group B (15%), and vs. Group E (1%). Age, number of pregnancies and miscarriages, or implantation rate in donors (327 women aged <35 years) were similar in all the five groups.*

Conclusions: *Endometrial receptivity is tolerant to a wide duration of E_2 treatment (until 2 months), while waiting for oocytes available for donation, but best results are achieved with a treatment range of 11 to about 40 days.*

KEY WORDS: Endometrial receptivity; hormonal replacement; oocyte donation; recipient.

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INTRODUCTION

Oocyte donation is an important tool for fertility in women affected by premature ovarian failure (POF) (1) or genetic disorders as well as in women who failed to respond to ovarian stimulation or to fertilize oocytes in previous IVF cycles (2).

One of the most important problems in an oocyte donation program is synchronization between donor and recipient. The implantation window (period of endometrial receptivity for the implantation of the blastocyst) in a normal ovulatory cycle is limited to a few days (17–19th day of the cycle) and depends on the sequential action of estradiol and progesterone on the endometrium (1). The most common method for synchronizing the recipient's endometrium with the donor's cycle is artificial preparation with sequential administration of exogenous estradiol and progesterone, imitating a natural cycle. The problem is that, owing to the low number of donors in countries like Italy, where pay donors are not allowed and where eggs only from women themselves undergoing an assisted reproductive treatment (ART) are allowed, one recipient might have to wait a long time until oocytes are available. This calls for flexibility in the length of estradiol treatment before progesterone is added when oocytes become available. Serhal and Craft (3) described a simplified approach using a fixed daily dose of 6 mg estradiol valerate up to 28 days.

Some authors (4–6) assessed endometrial receptivity with different estradiol treatment durations, but no conclusive results have as yet been provided. Navot (7) compared a short protocol of hormonal

manipulation of the endometrium (6 days of administration), with a long protocol (21–35 days of E_2 administration) and control group (14 days of E_2 administration). Midluteal and late luteal endometrial biopsies were morphologically similar in each group, showing no detrimental effect of both short and long estradiol treatment.

The aim of this study was to investigate if the duration of the estrogenic endometrial stimulation in the recipients can affect the pregnancy rate in an ovum donation program.

MATERIALS AND METHODS

Donors

Oocyte donors were 327 patients undergoing ART between 1995 and 1999, who wished to donate anonymously their excess oocytes. They were all aged <35 years, with a mean age of 30.9 years (range 21–35). Each donor and her male partner underwent a screening examination as previously described (8) and signed informed consent. Each recipient received oocytes donated by only one donor. Each donor donated oocytes to one or more recipients. In some cases, the same donor donated eggs to recipients of different groups.

No more than three embryos per IVF/ET were transferred, according to the rules established by the Italian Fertility Society (SIFES), with a view to reducing multiple pregnancies.

The superovulation protocol in all donors included the i.m. administration of either leuprolide depot (LA) (Enantone depot, Takeda, Catania, Italy) or triptorelin depot (Decapeptyl 3.75, IPSEN Spa, Milan, Italy) on Day 21 of the previous cycle. Four ampoules of FSH (300 IU) (Metrodin or Metrodin HP 75; Serono, Rome, Italy) were administered on Day 2 of the cycle for two days, followed by two per day (150 IU) for four days; then the dose was adjusted according to the individual response as estimated by E_2 assays and ultrasound scanning performed every other day until ultrasound examination detected two follicles 22 mm in maximum diameter. The transvaginal collection of oocytes was performed with ultrasound guidance 34 h after the administration of 10,000 IU hCG (Profasi HP; Serono; Rome, Italy).

Recipients

Five hundred and twenty women aged between 22 and 51 years were treated with a total of 835 transfer cycles; 310 were menopausal or with primary amenorrhoea (497 cycles) and 210 had functioning ovaries (338 cycles). Cyclic patients chose oocyte donation either because of their age, or because they had been "poor responders" in previous IVF cycles.

All patients underwent a preliminary blood, cardiographic, and pulmonary work-up. Hysteroscopy was used to confirm the presence of an adequate uterine cavity.

The male partner submitted a semen sample for sperm analysis. Both partners signed an informed consent form.

Before the transfer cycle, endometrial development was assessed in a mock cycle hormone replacement therapy (HRT) as previously described (8).

Each recipient received oocytes donated by only one donor. Recipients received no more than five eggs and no more than three embryos were transferred, with the aim of decreasing the incidence of triplets.

Each recipient received micronized 17β-estradiol (E_2) orally (Estrace, Mead Jonhson Laboratories, Evansville, IN), in a steadily increasing dosage from 2 to 6 mg daily over a period of time varying from 5 to 76 days depending on the patient (Fig. 1).

All the patients started with 2 mg/day of E_2 for six days, then the dose was increased to 4 mg/day. Such a dose was maintained until a donor was available; 2–4 days before donor's oocyte retrieval the E_2 dose was increased to 6 mg/day. Progesterone (P) supplementation either as injection of 100 mg in oil (Prontogest; Amsa, Florence, Italy) or as 600 mg micronized (Utrogestan; Piette, Brussels, Belgium) via vaginal route was started on the day of oocyte collection in the donor. Our previous studies (9,10) showed no differences in terms of pregnancy and implantation rates between patients using P in oil vs. vaginal micronized. In the transfer cycle, endometrium line thickness was checked on the first day of P, no supplementation being begun if the line was thinner than 8 mm or thicker than 12 mm. Menopausal women started estradiol immediately upon admission
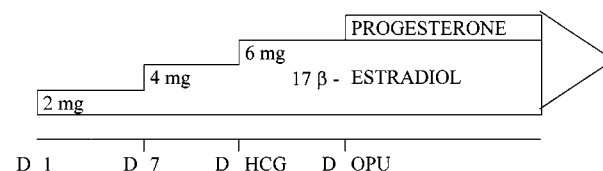

Fig. 1. Hormone treatment protocol in recipients. D HCG: day of HCG administration in donor; D OPU: day of oocyte collection in donor.

to treatment. Patients with ovarian function were desensitized with the i.m. administration of leuprolide acetate depot (Enantone depot, Takeda, Rome, Italy) or triptorelin depot (Decapeptyl 3, 75, IPSEN Spa, Milan, Italy) on Day 21 of the previous cycle.

All transfers were performed on the third day of progesterone administration and carried out into the uterus (11,12). In cases of pregnancy, both E_2 and progesterone were continued for 65 days after transfer.

All the recipients who started P after less than seven days of E_2 treatment, because a donor was suddenly available, increased the E_2 dose directly from 2 to 6 mg, one or two days before the donor's oocyte retrieval. In such patients transfer was performed only if a US scan showed an endometrial thickness of at least 8 mm, otherwise all embryos were frozen waiting for a next more suitable endometrial preparation.

Patients were retrospectively divided into five groups depending on the duration of E_2 administration. Group A (61 cycles of oocyte donation) was treated for 6–10 days before P administration; Group B (376 cycles) for 11–20 days; Group C (228 cycles) for 21–30 days; Group D (105 cycles) for 31–40 days; Group E (65 cycles) for >40 days before P administration (range 41–76 days).

Pregnancy was defined as the presence of one or more gestational sacs detected on a US scan performed at least 4 weeks after embryo transfer. Biochemical pregnancies (a rise of β-hCG with no further evidence of gestational sac on US scan) were not considered.

Statistical analysis used Student's t-test, χ^2 test, Fisher's exact test, and ANOVA as appropriate. A value of $p < .05$ was considered to be statistically significant.

RESULTS

Recipients

Mean age of the recipients was 41.3 ± 6.7 years. No difference was seen in mean age among the five treatment groups. The number of cycles cancelled because of breakthrough bleeding was 0 in Group A, 6 (1.6%) in Group B, 10 (4.2%) in Group C, 5 (4.6%) in Group D, and 5 (7.2%) in Group E; the difference is not significant.

A total of 835 transfers were performed; 1.8 ± 0.7 embryos were transferred per cycle, obtaining 224 pregnancies (pregnancy rate 26.8%), with 253 gestational sacs (16.7% implantation rate). Forty-five pregnancies ended in early abortion (20%), and one was ectopic (0.5%).

Pregnancy, implantation, and abortion rates in women with functioning ovaries were not different from the menopausal recipients (26%, 16.4%, and 23% vs. 27%, 17.1%, and 17%, respectively). Menopausal and cycling woman were uniformly distributed among groups. Table I shows the clinical results according to duration of estradiol treatment (upper panel: recipients). There was no difference between groups in the number of embryos transferred.

Table I. Results of Oocyte Donation According to the Length of E_2 Treatment

Groups	A (≤10 days)	B (11–20 days)	C (21–30 days)	D (31–40 days)	E (>40 days)
Recipients					
Age	41.4 ± 6.6	41.1 ± 6.8	40.9 ± 6.6	41.9 ± 6.6	42 ± 7.1
No. of transfers	61	376	228	105	65
No. of transferred embryos	1.9 ± 0.6	1.8 ± 0.6	1.8 ± 0.6	1.9 ± 0.7	1.7 ± 0.6
Pregnancies (%)	17 (27.9)	107 (28.5)	56 (24.6)	29 (27.6)	15 (23)
Miscarriages (%)	7 (41)**,*	16 (14.9)**	15 (26.8)	6 (20.6)	1 (6.7)*
Ectopic pregnancies (%)	0	0	1 (1.8%)	0	0
No. of gestational sacs	20	121	59	37	16
Implantation rate (%)	18	17.9	14.5	18.2	13.6
Donors					
Age	30.8 ± 2.6	30.9 ± 2.9	30.9 ± 2.8	30.9 ± 2.5	30.7 ± 3.5
No. of transfers	50	200	153	81	50
No. of transferred embryos	2.2 ± 0.5	2.2 ± 0.4	2.2 ± 0.6	2.2 ± 0.4	2.3 ± 0.5
Pregnancies (%)	17 (34)	61 (30.5)	42 (27.5)	29 (36.1)	15 (30)
Miscarriages (%)	3 (17)	4 (6.5)	3 (7.1)	2 (6.8)	0
Ectopic pregnancies	0	2 (3.2)	0	0	0
No. of gestational sacs	21	74	52	34	17
Implantation rate (%)	19.1	16.8	15.5	19.8	15.3

*$p = .04$; **$p = .02$.

No significant difference was seen in pregnancy and implantation rates between the five groups. There was a significantly higher number of miscarriages in Group A (41%), $p < .05$ vs. Group B (15%) and vs. Group E (1%).

Donors

Donors were divided in five groups matching each recipient with her own donor. Since in some cases one donor donated eggs to more than one recipient, it was possible for the same transfer cycle to be considered in different groups, but only once in the same group. Table I shows clinical results in donors (lower panel). Age of donors and number of embryos transferred were similar in the five groups. No significant differences arose in the number of pregnancies and miscarriages or in implantation rates between the groups.

DISCUSSION

In an oocyte donation program in which donors are patients undergoing an ART cycle, the number of eggs available for donation is not very high. In order to give recipients the best chance to obtain oocytes, it is very important to adopt a very flexible protocol of HRT that would allow recipients to be ready for synchronization for a long period of time.

Younis (5), using a simplified method of endometrial preparation with 4 mg/day micronized estradiol per os for 5–35 days, demonstrated that the optimal duration of estrogenic endometrial stimulation is 12–19 days (PR 52%). When a treatment lasted less than 12 days and more than 19 days, PR dropped to 7.7%. Michalas (13) reports a higher pregnancy rate with a micronized E_2 therapy between 6 and 11 days. Over 11 days of unopposed E_2 treatment, PR drops dramatically. Both reports contrast with Navot's studies (7) that compared a short protocol (6 days of E_2 administration) with a long protocol (21–35 days of E_2 administration) and control group (14 days of E_2 administration). Midluteal and late luteal endometrial biopsies were morphologically similar in each group, showing no detrimental effect of either short or long estradiol treatments. Progesterone addition seems to allow normal endometrial maturation regardless of the length of estradiol therapy. Younis (5,14) explains his results by suggesting that normal endometrial morphology does not always mean normal endometrial receptivity.

Our results show that a good pregnancy rate can be achieved either with short (<10 days) or long (even more than 40 days) E_2 treatment. Above 40 days pregnancy and implantation rates show a tendency of a little decrease, but pregnancies are still possible after about 60 days of E_2 treatment.

This confirms the findings of previous studies (2,4,15). Navot (15) compared a short (5–10 days of transdermal E_2 administration) with a long (21–42 days of transdermal E_2 administration) protocol and found similar PR regardless of endometrial stimulation. However, no data about treatment duration over 6 weeks are available from Navot's studies.

Yaron (4) shows no significant difference in PR (ranging from 19% to 27%) when 6 mg/day of E_2 valerate are administered for 5–35 days. After 35 days of treatment, PR drops sharply to 7%. As in our study the number of subjects in this group is low and this may explain why the difference is not significant, though it should be noted that in the present study the decrease is very small and the number of observations in the longest treatment is wider.

Our study agrees with the work of Remohi (6), who is, to date, the one treating recipients with the longest duration of E_2 therapy. Remohi achieved good results even with E_2 therapy lasting more than 65 days and demonstrated that pregnancy is possible with 80 or 100 days of estradiol valerate treatment before adding progesterone. Remohi, however, does not consider in his work cycles shorter than 10 days.

In our series the longest duration of E_2 treatment was 76 days and the number of patients receiving E_2 for more than 45–50 days is very low compared with other treatment groups, because of our choice in previous years to discontinue treatment, adding progesterone in order to induce withdrawal bleeding, whenever there were no eggs available after about 45–50 days of treatment. One pregnancy did, however, occur after 64 days of E_2 administration, one after 59 days, and two after 53 days, while the shortest treatment period for achieving pregnancy was 6 days.

In our study the incidence of cycle cancellation for breakthrough bleeding was low in all groups. There was a tendency to increase after 40 days of therapy, but the increase is not significant, also because of small number of cases.

All the above reports evaluated relatively small groups of patients, especially Younis (5) and Michalas (13), a factor which might explain the discordance of our findings with the two other studies. Moreover Younis administered a lower dose of both E_2 (4 mg/day) and P (50 mg/day) than we did.

The number of patients treated by Navot (15) and Remohi (6) was also lower than that in our series. The only study similar to ours in terms of observations is the one reported by Yaron (4), though here the number of treatments over 35 days was lower.

A crucial point in our study is the high abortion rate in the shortest E_2 group notwithstanding the high pregnancy and implantation rates. This supports the finding of Navot (15), who reports a 52.9% early pregnancy loss in the short protocol (5–10 days of transdermal E_2 administration) compared with 18.8% in the long protocol (21–42 days of transdermal E_2 administration), suggesting an adverse effect of the short cycle on endometrial functional receptivity. It was suggested (15) that exposure of endometrium to estradiol for a short time might stimulate more surface epithelium (favoring embryo attachment) than stromal compartment (necessary for sustained implantation). In contrast, prolonged estrogen stimulation might uniformly affect both epithelium and stroma, providing an optimal environment for sustained implantation.

Only one miscarriage was seen in recipients receiving E_2 for more than 40 days. It should be noted that no miscarriages were found in donors of Group E, and hence might be related not only to endometrial receptivity, but also to embryo quality.

The age of recipients, and not just the age of donors, has been shown to influence the outcome of ovum donation, though this question is highly controversial. Sauer (16,17) found no difference in pregnancy rate after oocyte donation in woman <40 years old compared with those >40 years old. On the other hand Flamigni (8) found a lower PR in recipients between 40 and 49 years than in their oocyte donors or younger recipients. This finding was confirmed by a study comparing recipients <40 years and recipients between 40 and 49 sharing oocytes from a single donor (10). Each donor donated oocytes to one recipient of both age groups: pregnancy and implantation rates in younger recipients were similar of those of donors, while in older recipients it was significantly lower highlighting the influence of uterine age on implantation. A recent large study about more than 1,000 oocyte donation cycles in Israel (18), showing a significant age-related decrease in pregnancy rate in recipients, further supports these findings.

In the present study, the age of both donors and recipients was homogeneous between the different groups, thus excluding the influence of this confounding factor on clinical results. Long-term unopposed E_2 treatment may give rise to concern about cardiovascular risk, haemocoagulative accident, or endometrial cancer. No adverse effects were recorded in our patients, supporting the conclusion of Remohi (6) that long follicular phase E_2 replacement therapy is a safe procedure. As regards the risk of endometrial cancer, Remohi stated that performing an endometrial biopsy during a mock replacement therapy cycle before enrolling patients in the ovum donation program and adding progesterone for about seven days before stopping treatment would be safe enough (6). This is a procedure also supported by other authors (19,20). All the patients of our study underwent an endometrial biopsy during the mock cycle (8). Only patients with no sign of hyperplasia were accepted in our program.

In conclusion, our study shows that endometrial receptivity is tolerant to a wide duration of E_2 treatment. A pregnancy can begin after a very short duration of E_2 treatment (e.g., 6–7 days) and even a woman waiting for oocyte donation for a longer period, up to 2 months, may have an acceptable chance of bearing a child. However, considering the high abortion rate in the shortest E_2 group and the tendency of increase of breakthrough bleeding after more than 40 days of therapy, treatment duration from 11 to about 40 days seems to be preferable.

This wide interval allows a satisfactory synchronization between donors and recipients in an anonymous oocyte donation program.

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