

## The use of a shared donor oocyte program to evaluate the effect of uterine senescence\*

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**Objective:** To determine if there is reduced uterine receptivity after age 40 by the comparison of pregnancy rates (PRs) of donor oocyte recipients  $\geq 40$  years to those under age 40.

**Setting:** In vitro fertilization-ET facility of a university-based practice, Cooper Institute for In Vitro Fertilization.

**Patients:** All patients registering for the shared donor oocyte program from November 1990 to September 1992. Most recipients were in ovarian failure.

**Interventions:** Donors were treated with luteal phase leuprolide acetate (LA) and gonadotropins; recipients were treated with oral  $E_2$  in graduated doses and 50 mg IM daily P. Endometrial thickness was considered in the decision to continue with transfer or to freeze all embryos.

**Main Outcome Measures:** Pregnancy rates per transfer in recipients and live birth rates according to age  $\geq 40$  or  $< 40$ .

**Results:** The clinical PR per transfer was 29.2% for the younger women and 25.4% for the older recipients. The live birth rate was 29.2% for the younger women and 22.4% for the older recipients.

**Conclusions:** These data support the conclusion that, if there is a decline in uterine receptivity for embryo implantation with advancing age, it is at least remediable with hormonal adjustments. Fertil Steril 1994;61:252-6

**Key Words:** Age, uterine senescence, shared donor oocytes

There have been previous animal studies showing a marked decrease in deliveries when embryos from young animals were transferred to older recipients; the rates dropped in mice from a 48% delivery rate in young versus 14% in older mice (1), 49% to 8% in the hamster (2), and 50% to 2% in the rabbit (3). Similarly, lower pregnancy rates (PRs) have been reported in patients  $\geq 40$  years of age compared with younger women when donor oocytes

from young women were used. Abdalla et al. (4) reported their experience in 100 cycles of oocyte donation and found a 16% PR in the older patient versus 36% in those  $< 40$ . Another study by Levran et al. (5) found in their donor oocyte program a higher PR in recipients  $< 33$  (31%) compared with older recipients (14%) and Meldrum (6) found in recipients given 50 mg IM P in the luteal phase, a PR of 43% in those  $< 40$  but only 8% in those  $\geq 40$  years of age.

One previous study found that if the endometrial thickness of the recipient at the time of the donor's hCG injection was  $< 10$  mm only 9% (2 of 22) conceived compared with 38.7% (14 of 36) when  $> 10$  mm (7). Check et al. also found a lower PR in donor

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oocyte recipients  $\geq 40$  (2 of 23, 8.5%) compared with recipients  $< 40$  (14 of 55, 25.4%) (Check JH, Choe J, Barnea ER, Adelson HG, abstract); the endometrial thickness was found to be  $< 10$  mm in 61% of those  $\geq 40$  and was thin in only 29% of those  $< 40$ . The study presented herein evaluated PRs in donor recipient cycles according to age of recipient where no difference in therapy (except adjustment for endometrial thickness) was given to older versus younger recipients.

## MATERIALS AND METHODS

### Shared Oocyte Program

The shared oocyte program provided recipients with 50% of the retrieved oocytes from infertile women who themselves require IVF-ET and are willing to share their oocytes in return for financial assistance in paying for their own IVF cycle. All patients  $< 40$  years old requiring IVF-ET were eligible to be donors; the physical characteristics, education, medical history, family history, social history, cause of infertility, previous fertility status, data whether they have had IVF-ET before and whether pregnancies ensued (in the patient or another recipient), percent fertilization, and quality of embryos in previous IVF cycles were listed.

### Stimulation Protocols for Donors and Recipients

For controlled ovarian hyperstimulation of the donors, the luteal phase leuprolide acetate (LA) suppression of gonadotropins followed by hMG 300 IU daily was used (8). The recipients began oral  $E_2$  in graduated doses beginning on the 6th day of the donor's LA therapy. The details of  $E_2$  replacement previously have been described (9). When the donor had two dominant follicles reaching an average of 20 mm and a serum  $E_2$  of  $\geq 800$  pg/mL (2,936.0 pmol/L), the endometrial thickness of the recipient was measured. If the thickness was  $\geq 10$  mm, the donor was given 10,000 units of hCG and the retrieval was scheduled. If the recipient's endometrial thickness was  $< 10$  mm, the donor was stimulated further, while the recipient was given further supplementation. If the recipient responded, the donor's retrieval and both transfers were scheduled. If the recipient failed to respond, the retrieval would still take place, but the recipient's embryos would be cryopreserved and transferred on a succeeding cycle where adjustments were made to increase her oral  $E_2$  levels. If, despite efforts to improve the en-

dometrial thickness, the 10-mm levels could not be reached, the frozen ET was performed as scheduled. Patients had the right to proceed with a fresh ET without cryopreservation, even with a thinner endometrium.

### Cryopreservation Methods

All embryos were cryopreserved using the modified one-step method originally designed for bovine morulas (10). Two pronuclear (2PN) and cleaved embryos were equilibrated in 1.5 M 1,2 propanediol and frozen in 0.25-mL straws in an alcohol bath rate controlled freezer as previously reported (11, 12). All cryoprotectant solutions were phosphate buffered and contained 3 mg bovine serum albumin/mL. The straws were preloaded with 0.120 mL of 1.08 M sucrose followed by a 1-cm air column and then the embryo in 0.020 mL of 1,2 propanediol. The freezing program started at  $-6.0^\circ\text{C}$  at a ramp rate of  $-0.4^\circ\text{C}/\text{min}$  down to  $-40^\circ\text{C}$ . Straws were then stored in liquid nitrogen. The cryoprotectant was diluted out in one step after thawing by vigorously shaking the straw to mix the sucrose with the embryo. When transfers were to be cancelled because of inadequate endometrial thickness, the zygotes were frozen at the 2PN stage.

### Patient Inclusion

One hundred fifty-five donor-recipient cycles in the shared oocyte program at the Cooper Institute for IVF between November 1990 and September 1992 were included in this study. Patients were divided into two groups based on the age of the recipient at the time of transfer. Group 1 consisted of 67 recipient cycles in which the recipients were  $\leq 39$  years of age. Group 2 consisted of 88 cycles in which the recipients were  $\geq 40$  years of age at time of transfer. The distribution of infertility factor of donor, mean age of donors, and mean number of embryos transferred were compared in the two recipient age groups to ensure against any bias in the groups.

### Outcome Measures

Clinical PR per transfer and live birth rates per transfer were computed and compared by age of recipient. A clinical pregnancy was defined as a pregnancy in which there was ultrasound evidence of a gestational sac.

## Statistical Analysis

Chi-square analysis was used to test the hypothesis that the PR and live birth rate do not differ by age of recipients. The *t*-test for independent groups was used to compare mean donor age and mean number of embryos transferred in the age groups. All tests were performed at the 0.05 level of significance.

## RESULTS

Group 1 consisted of 67 donor oocyte recipients who were < 40 years of age (mean age  $34 \pm 4$  years). Oocytes were received from donors who had a mean age of  $32 \pm 4$  years; 54% of the donors had tubal factors, 12% endometriosis and/or pelvic adhesions, 12% male factor, 10% were unexplained infertility, and 12% had miscellaneous ovulatory dysfunction (e.g., the lutenized unruptured follicle syndrome). Group 2 consisted of 88 recipients age 40 or older (mean  $\pm$ SD;  $44 \pm 3$  years). They received their oocytes from donors with a mean age of  $32 \pm 5$  years of age. Fifty-three percent of these donors had tubal factor, 10% endometriosis/pelvic adhesions, 17% male factor, 5% unexplained infertility, and 15% miscellaneous ovulatory dysfunction. There was no difference in the distribution of donor's infertility factors in the two age groups ( $\chi^2$ ,  $P > 0.05$ ).

After retrievals, transfers were cancelled for 13 recipients in group 1 and 21 recipients in group 2. There were three transfers in group 1 that were cancelled due to no fertilization; in the remaining 10 cycles, all embryos were frozen, 9 because the recipients' endometria and hormonal levels were not adequate, 1 because the recipient decided to use a host womb. In group 2, 21 transfers were cancelled, 5 because of poor fertilization (0 or 1 embryo available) and 16 in which all embryos were frozen: 2 waiting for host womb, 1 became pregnant on own, 13 because of poor endometrial and hormonal levels.

The mean number of embryos transferred were  $4.9 \pm 4.1$  in group 1 and  $4.3 \pm 1.3$  in group 2 (*t*-test, not significant). The clinical PR/transfer was 29.6% for the younger recipient and 25.4% for the older recipient. The live birth rates were 29.6% for younger recipients and 22.4% for older recipients (Table 1). Chi-square analysis found no difference in these rates at the 0.05 level of significance. The corresponding rates for donors in each recipient group were 15.1% clinical and 11.3% live birth for

**Table 1** Comparison of Pregnancy Rates by Age of Recipient

	Group 1, age < 40*	Group 2, age $\geq$ 40*
No. of retrievals		
Recipients	67	88
No. of transfers	54	67
Clinical pregnancies	16 (29.6)	17 (25.4)
Live birth	16 (29.6)	15 (22.4)
Donors		
No. of transfers	53	63
Clinical pregnancies	8 (15.1)	9 (14.3)
Live births	6 (11.3)	5 (7.9)

\* Values in parentheses are percentages.

group 1 and 14.3% clinical and 7.9% live birth for group 2.

The recipient decided to have the transfer even though her endometrial thickness was <10 mm in 11 of the fresh transfers (8 in group 1 and 3 in group 2). The PRs for these cases were 12.5% for group 1 and 0% for group 2.

There were 22 patients who cryopreserved all their embryos and 18 had a subsequent frozen ET. There was one clinical pregnancy but no live births in nine group 1 frozen ETs, and one ectopic and no clinical pregnancies in nine group 2 patients. There are insufficient data at this time to make any inferences.

## DISCUSSION

The data from this larger study no longer support the previous conclusions from our preliminary study where a reduced PR was seen in women  $\geq$  40 years of age, although, possibly, the change in data was related to the change in protocol related to endometrial thickness. Recently, there have been data provided by other groups demonstrating no decrease in their PR for older recipients, leading to the conclusion that reduced fecundity with advancing age is solely related to a decline of oocyte quality rather than uterine senescence (13, 14).

The possibility exists, however, that the decline in fecundity with advancing age is related to both decline of oocyte and uterine quality; only the former is not correctable but the latter is more remediable. Chetkowski et al. (15) found that in women  $\geq$  40 years of age embryo viability dropped from 0.27 to 0.16 and uterine receptivity decreased to 0.29 in the older women from 0.42 in the younger (<40) group. Meldrum (6) has hypothesized that

one of the most important aspects of the aging uterine factor may be reduced sensitivity to P. His group found that when recipients  $\geq 40$  years of age were treated with the same P regimen of 50 mg IM daily there was a much reduced PR compared with women  $< 40$  given the same P dosage (8% versus 43%); however, when females  $\geq 40$  were given 100 mg IM, the PR (46%) increased to the same level as the younger women given 50 mg IM (6). There are data to support a higher incidence of delayed endometrial maturation in women over age 35 stimulated for IVF (15).

The fact that our study used only the smaller dose of intramuscular P and still showed the same PR as the younger group does not negate Meldrum's hypothesis; in fact he states that "it is also possible that P receptivity could be increased in some oocyte donation recipients by more prolonged or elevated  $E_2$  stimulation" (6). Before we made adjustments for endometrial thickness by raising  $E_2$  dosage or prolonging  $E_2$  exposure, we did, in fact, find reduced PRs in the older patients (Barnea ER, Peymer M, Jairaj P, Check JH, abstract). One may hypothesize that the higher dose of P may overcome, to some degree, inadequacies as detected by thinner endometria.

Ben-Nun et al. (16) published data suggesting that giving 100 mg IM P daily could increase the PR in women of all ages undergoing IVF. Although we were unable to corroborate these data (17), the possibility exists that the younger recipients may have shown a superior PR if they had been treated with higher doses of P in the luteal phase.

Even with the use of oral  $E_2$  for longer time periods or increasing the dosage, there were still more cancellations in the older group for inadequate thickness (22 of 104 or 21.1%) than in the younger group (15 of 138 or 10.8%). The data from Sauer (13) and Meldrum (6) are impressive using the 100 mg IM dose of P and presently all new recipients  $\geq 40$  years are given the higher dose of P in our program. The Cooper Institute for In Vitro Fertilization is considering a study of all recipients where the higher and lower dosages of intramuscular P would be randomized and the effect of P dosage according to age would then be determined.

The mean age of the recipients in group 1 was 34 and for group 2 was 44. However, Levran et al. (5) found a lower discriminating age of 33 in that only 14 of 97 (14%) conceived when  $\geq 33$  years of age compared with 16 of 58 (31%)  $< 33$ . Perhaps some decline in uterine receptivity would have been dem-

onstrated in our study if we only had compared women  $< 33$  with the older age group.

There are data suggesting that there is a higher PR with oocyte donation when the donor is under age 30 (18). We considered the possibility that embryos derived from younger oocytes taken from very fertile women are able to form embryos that can somehow overcome the uterine deficiencies of the older endometrium. For this reason we only included for this study data from our shared oocyte program where the oocytes were taken from infertile women. The average age of the donors was between 31 and 32 years and was the same for group 1 and group 2. We have demonstrated no difference in PRs in recipients even when they receive oocytes from donors age 35 to 39 (Check JH, Choe J, Adelson H, Fisher C, Callan C, abstract). There was no higher spontaneous abortion rate in the older recipient as evidenced by similar viable PRs and live birth rates. These data support the conclusion that if there is a decline in uterine receptivity for embryo implantation with advancing age, it is at least remediable with hormonal adjustments.

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