The effect of age on in vitro fertilization outcome: is too young possible?

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Received: 26 August 2010 / Accepted: 15 October 2010 / Published online: 2 November 2010
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Abstract

Purpose The negative correlation between fecundity and age in women has been extensively documented although data on reproductive performance in very young women is sparse. The objective of this study was to determine whether age ≤ 25 years has an impact on reproductive outcome in women undergoing IVF-ET.

Methods IVF outcome in 85 infertility patients aged 19–25 years was compared to that in 69 infertility patients aged 30–35 years. Primary outcomes included fertilization rates and embryo quality. Secondary outcomes were clinical pregnancy and miscarriage rates.

Results The young patients (≤ 25 years) demonstrated a lower fertilization rate, and reduced number of top quality embryos. Although clinical pregnancy, and implantation rates were similar to their older counterparts (30–35 years), the young women had a significantly higher miscarriage rate.

Conclusion Our results demonstrating poorer reproductive performance in very young patients were surprising and need further investigation.

Keywords In vitro fertilization · Young patients · Pregnancy · Egg donor · Reproductive outcome

Introduction

It is well known that the pregnancy rate is inversely related to the age of the female partner. Some explanations for this include diminished ovarian reserve, increased rate of aneuploidy, decreased frequency of sexual intercourse, diminished desire for childbearing, and increased rate of spontaneous abortion. Among infertile women undergoing IVF treatment, advanced maternal age is also associated with lower fertilization rates and higher risk of chromosomal abnormalities [1].

Despite our knowledge of the impact of advanced maternal age on fertility, the quality of oocytes produced by very young patients or by young donors following IVF cycles has not been carefully investigated. It is generally assumed that young women are fundamentally the best category of patients for infertility treatment or for oocyte donation and should produce normal healthy eggs with low rates of chromosomal abnormalities. Recently, a number of reports have shown a wide range of chromosome abnormalities in the embryos created from the oocytes of young egg donors [1–3]. Moreover, the frequency of vanishing embryos in patients with multiple pregnancies from oocyte donation is relatively high. The vanishing embryo phenomenon is defined as the early resorption, in the first trimester, of one or more embryos in a multiple gestation, after confirmation by transvaginal ultrasound [4].

The purpose of this retrospective cohort study was to determine whether young age (25 years and younger) was associated with decreased oocyte and embryo quality and pregnancy outcome.

Capsule Our results demonstrated that very young women had poorer reproductive performance compared to patients in their early thirties.

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Materials and methods

Patients

Data from patients who underwent IVF/ICSI over 15 years from 1994 to 2009 were analyzed retrospectively. The records of 57 infertility patients aged 19–25 years (61 cycles) and 28 oocyte donors aged 20–25 years (31 cycles) were collected and reviewed. Sixty-nine infertility patients aged 30–35 years (71 cycles) with similar infertility diagnosis and IVF/ICSI treatment in the same time frame as the young patients served as matched controls. Patient demographics, cycle characteristics and cycle outcome were compared among the three groups.

Semen analysis and sperm preparation

Semen samples were collected after 2–3 days of sexual abstinence. Specimens were allowed to liquefy for up to 1 h after collection and the volume, appearance, pH, and semen viscosity were determined. Manual semen analysis was performed according to WHO guidelines to determine sperm concentration and motility [5]. A 5- μ L aliquot of liquefied semen was loaded on a microcell counting chamber (Conception Technologies, San Diego, CA) and examined under $\times 200$ magnification. Sperm concentration was expressed as $\times 10^6$ /mL semen, whereas motility was expressed as a percentage. Progressive sperm motility was defined as grade A+B (rapid progressive and sluggish progressive). In this study, normal values were: sperm concentration equal to or greater than 20×10^6 /mL semen, motility 50% or greater, and normal sperm forms 30% or greater.

Discontinuous density gradients were prepared by overlaying 1.5 mL of 80% Pure Sperm (Nidacon International, Gothenburg, Sweden) solution with 1.5 mL of 40% solution. Aliquots of 1.5 mL of the liquefied semen were carefully layered over the uppermost Pure Sperm layer. The gradient was centrifuged at $300 \times g$ for 20 min at room temperature. The resultant pellet was resuspended in 5 mL human tubal fluid supplemented with 5% human serum albumin (HTF-HAS), and centrifuged at $300 \times g$ for 7 min. The supernatant was discarded and the pellet was resuspended in 0.5 mL of HTF-HSA for oocytes insemination.

Ovarian stimulation and oocyte retrieval

The patients were prepared using standard long protocol of pituitary desensitization, with the GnRH agonist (Suprefact, Aventis Pharma, Laval—Quebec, Canada) starting in the mid-luteal phase. Controlled ovarian stimulation was started utilizing recombinant FSH following confirmation of pituitary desensitization. The starting dose for FSH was

150 to 300 IU depending on the age of the patient, history of ovarian stimulation, and the number of basal antral follicles seen on ovarian transvaginal ultrasound scans. Follicular growth was monitored with serial ultrasound scan and serum estradiol levels. When the maximum diameter of two or three leading follicles exceeded 18 mm, 10,000 IU human chorionic gonadotropin (hCG, Profasi, Serono Canada, Oakville, Ontario, Canada) was given subcutaneously. Transvaginal ultrasound-guided oocytes retrieval was performed 36 h after hCG administration. Follicles were aspirated into heparinized modified (HEPES buffered) human tubal fluid (mHTF, Irvine Scientific, Santa Ana, CA). Oocytes were collected from follicular fluid and washed in fresh equilibrated HTF medium supplemented with 10% synthetic serum substitute (SSS, Irvine Scientific, Santa Ana, CA) and incubated at 37°C in 5.5% CO₂ in humidified air until denuding for ICSI or insemination for IVF.

IVF and ICSI procedures

For IVF, 3 to 4 oocytes were placed in 1 mL HTF supplemented with 10% SSS (HTF-SSS) in a center well culture dish (Becton Dickinson, Franklin lakes, NJ), and 50,000 motile washed sperm per oocyte was added to the dish. For ICSI procedure, denuding was performed 2–4 h following retrieval, using 80-mIU/mL hyaluronidase (Sigma chemical Co., St Louis, MO) in HTF-HSA for 30–45 s. The oocytes were then washed in three consecutive washes of 37°C HTF/SSS. ICSI was performed as described earlier [6]. Approximately 18 h after injection or insemination, oocytes were checked for signs of fertilization (two distinct pronuclei and two polar bodies). At 40–42 h embryos that had cleaved were identified and graded using criteria based on blastomere number, symmetry, and degree of fragmentation on a scale of 1–5 where grade 1 represents the highest quality. The majority of embryo transfers was performed on day three-post retrieval using the Cook catheter (Cook Australia, Queensland 4113 Australia). Embryos of the highest quality at the 6–8-cell stage with no or minimal fragmentation were transferred when available. Any excess cleaving embryos with more than 4-cell and less than 25% fragmentations were cryopreserved. A pregnancy test was performed 14 days after ET and clinical pregnancy was declared upon finding a gestational sac within the uterus on ultrasound.

Primary and secondary outcome measures

The primary outcome measures were fertilization rate, degree of embryo fragmentation, and mean embryo scores. The secondary outcome measures were pregnancy and implantation rates, and pregnancy loss rates.

Table 1 Patient demographics of study groups

Variable	Young patients	Young donors	Control	P-value
Number of cycles	61	31	71	
Number of Patient	57	28	69	
Patient Age (Mean±STD)	23.9±1.26	22.9±1.36	32.7±1.43 ^a	0.0001
Age range	19–25	20–25	30–35	NS
Serum E ₂ level on hCG day (pg/ml) (Mean±STD)	7515.9±5791.8	6029.4±4567	7316.5±4060.9	NS
Endometrial thickness (mm) (Mean±STD)	1.03±0.21 ^a	0.96±0.38	1.03±0.24	NS
No. of follicles ≥14 mm on hCG day (Mean±STD)	11.9±6.4	10.9±4.5	11.3±4.3	NS

^a Significantly different from other groups

Statistical analysis

All variables were stored in an Excel database and reported in order to describe the sample size as mean ± the standard deviation for continuous and as frequency tables for discrete variables. Data were analyzed using SPSS program (with the confidence interval of 95%, square=5%). For continuous outcomes one-way ANOVA and for categorical outcomes Chi-square test was used.

Results

Patient cycle characteristics are presented in Table 1. As noted earlier, the underlying diagnoses leading patients into infertility treatment, and the time of cycle were matched between study and control groups. Peak E₂ levels and number of ovarian follicles ≥14 mm at the time of hCG administration were similar between three groups. Semen characteristics of all groups are shown in Table 2. Although one more oocyte was retrieved in the control group as compared to young patients and donors, the number of mature oocytes (MII) was similar among the three groups. Significantly lower fertilization following IVF was observed in young patients compared to controls (Table 3) which seems to be at least in part due to the poor quality of oocytes in young patients and donors (Fig. 1a and b). Abnormal fertilization with one or 3 pronuclei was significantly higher in young patients and donors compared to the control group.

The number of cleaved embryos was significantly lower in young patients and donors compared to the controls. Embryo development and day-3 embryo quality was

superior in the control group and significantly lower quality embryos were transferred in young patients compared to the control group (Fig. 1c). In 6 cycles (10%) of young patients there was no embryo transfer, in 2 cases due to high OHSS risk, as well as 2 cases of failed fertilization, and 2 cases of embryo development arrest at 2PN. In addition, two cycles in the donor group had no transfer due in one case to failed fertilization and in the other to OHSS risk. A significantly lower percentage of cycles in young patients and donors resulted in cryopreservation compared to the controls (39.3% and 54.8% vs 80% respectively).

A comparable positive biochemical pregnancy test was achieved in the two study groups and controls (Table 4). Although the pregnancy rates were similar in the controls and the young patients or donors (34.3%, 29% and 27.6%, respectively) as were the implantation rates (19%, 15.3% and 13%), the miscarriage rates were significantly higher in young patients and donors compared to the controls (37.5%, 37.5% and 16.7%, respectively). These pregnancy rates were achieved in the three groups with the transfer of an average of 2.6 to 3.1 because the poor embryo quality in some of the patients mandated transfer of multiple embryos.

Discussion

The present study demonstrated that some young infertility patients and young oocyte donors produce lower quality oocytes and embryos as compared to their older counterparts. Because of the large number of oocytes retrieved in these young women, enough embryos were available to transfer, but the average quality of embryos and the number

Table 2 Semen characteristics in all groups

Variable	Young patients	Young donors	Control	P- value
Volume (mL)	2.6±1.5	3.1±1.3	2.9±1.6	NS
Count (M/mL)	21.7±27	63.8±36.1 ^a	30.7±36.6	0.001
Motility (%)	31.3±22.5	49±18.8 ^a	35.2±19.3	0.01
Morphology (%)	14.7±12.3	49.4±30.3	26.1±15.5	NS

^a Significantly different from other groups

Table 3 Cycle characteristics of study groups

Variable	Young patients	Young donors	Control	P- value
No. oocyte retrieved (Mean±STD)	12.3±6.9	12.7±7.4	13.9±6	NS
No. mature (MII) oocytes	9.4±6.2	9.4±5.8	8.9±6.1	NS
Normal fertilization in IVF (%)	34/114 (30) ^a	84/157 (53.5)	111/194 (57.2)	0.008
Normal fertilization in ICSI (%)	317/529 (60)	180/221 (81.4)	442/620 (71.3)	NS
Abnormal fertilization (1 and 3 pronuclei) (%)	41/643 (6.4)	28/378 (7.4)	4/814 (0.5) ^a	0.01
No. cleaved embryos (Mean±STD)	5.48±4.3 ^a	6.74±3.9	7.65±3.6	0.005
No. embryo Transferred (Mean±STD)	2.6±1.02	2.6±0.77	3.1±1.2 ^a	0.02
Embryo cell number at ET (Mean±STD)	6.75±1.4	6.9±1.5	7.28±1	NS
Transferred embryos fragmentation (Mean±STD)	2.1±0.83 ^a	1.7±0.66	1.6±0.66	0.003
No. frozen embryos (Mean±STD)	2.5±4.4	3.5±4.7	3.5±3.7	NS

^a Significantly different from other groups

of cycles with surplus embryos for cryopreservation was lower in the two groups of young women resulting in an overall poorer cycle outcome compared to the older control group. A common marker of embryo viability and developmental potential is the cleavage rate and degree of fragmentation. Embryos with slow development and high degree of fragmentation are generally thought to have a lower implantation potential than embryos with good morphology. The actual cause and mechanism of fragmentation is still not completely understood [7] but the amount of fragmentation on the third day of culture may be directly associated with chromosomal abnormality, and about half of the fragmented embryos are aneuploid [8]. The embryo quality mostly determines the number of embryos for transfer. Normally, if only low-quality cellular-stage embryos are available, a larger number is selected for embryo transfer.

It has become increasingly clear that diminished oocyte quality is the likely cause of reproductive aging and decreased pregnancy and live birth rates. The aging process is a complex phenomenon, driven by as yet incompletely

described genetic program(s). While a multitude of factors is thought to modulate cellular and organismal life span, only a few have been well defined. These include impaired mitochondrial function, genomic instability, and oxidative stress. Therefore, it is possible that as women and oocytes age and the mitochondrial energy production decreases, that many of the processes of oocyte maturation, especially nuclear spindle activity and chromosomal segregation, become impaired [9, 10]. The result is the increased rate of aneuploidy, especially trisomies observed in older women. Embryo chromosome studies show high rates of abnormalities, above 50% of embryos studied, in patients aged 35 and older.

The lower reproductive performance in young women compared to the older control group in the present study is surprising. Supraphysiologic E₂ levels following a high response to superovulation treatment is considered detrimental to embryos and to implantation [11, 12]. However, the mean value of E₂ in the young groups was similar to that of controls. Rodriguez-Gonzalez et al. found that the

Fig. 1 Retrieved oocytes
a from a young infertility patient
b from a young egg donor, and
c embryo quality on day-2 post
 retrieval in a young patient

Table 4 Pregnancy outcome among groups

Variable	Young patients	Young donors	Control	P- value
Positive beta hCG (%)	22/55 (40)	9/29 (31)	26/70 (37)	NS
Clinical Pregnancy per ET (%)	16/55 (29)	8/29 (27.6)	24/70 (34.3)	NS
Miscarriage rate (%)	6/16 (37.5)	3/8 (37.5)	4/24 (16.7) ^a	0.0015
Delivery rate (%)	10/55 (18.2)	5/29 (17.2)	17/70 (24.3)	NS

^a Significantly different from other groups

incidence of vanishing embryo phenomenon in oocyte donation pregnancies was significantly higher than in IVF or ICSI pregnancies [4]. They were uncertain about the explanation for these differences, although they did observe a higher multiple pregnancy rate in oocyte donation (68.7%).

A possible explanation for our observations is the rapid rate of follicle atresia that occurs as a result of programmed cell death from 5 months gestation to at least the age of puberty (reviewed in [13]). This accelerated ovarian loss slows down by the late 20 and early 30s. It is possible that the rate of follicular atresia is still relatively high in some women in their early 20s and that the controlled ovarian stimulation and oocyte retrieval results in collection of a large proportion of oocytes that have already activated a cell death program. These oocytes would be anticipated to exhibit lower fertilization rates and delayed or arrested embryo development and increased fragmentation rates. We believe this is the most logical explanation for poorer reproductive performance seen in very young women undergoing superovulation and ART.

Another possibility for the higher miscarriage rate we found in young patients and young donors in the present study is a higher rate of oocyte chromosomal abnormality. This hypothesis is supported by recent reports that a high rate of chromosomal aneuploidy including both trisomy and monosomy is present in embryos of young women. The proportion of abnormal embryos varied significantly between individuals and could not be predicted based on embryo morphology or on treatment cycle day-3 FSH levels. These results may explain why certain egg donors have poor pregnancy rates despite satisfactory ovarian response, oocyte quantity, and embryo morphologic appearance. [14]. In their recent study on the rate of chromosome abnormalities in embryos from young egg donors, Munne et al. have reported that on average, only 43% of the embryos from young egg donors were chromosomally normal. There was considerable variation between donor cycles, with almost one-third having less than 30% normal embryos. The results can explain why some egg donors are successful whereas others are not, and may also show that a policy of PGD for first time egg donors may be appropriate [15].

Our results demonstrating poorer reproductive outcome in young patients and egg donors compared to those in 30–35 years old control group are surprising since similar

stimulation and oocyte retrieval numbers were observed in all women. We speculate that some very young women may be on the shoulder of the decreasing curve of high follicle atresia rates seen prior to puberty resulting in higher rates of programmed cell death in their oocytes. In addition, high rates of oocyte aneuploidy have been reported in some young women suggesting that it may be reasonable to consider chromosome analysis of the embryos from young patients and young donors. This suggestion is controversial since current thinking is that young women and egg donors should have the least aneuploidy and best pregnancy rates. Our present observations suggest that this assumption may be incorrect.

References

1. Bentov Y, Esfandiari N, Burstein E, Casper RF. The use of mitochondrial nutrients to improve the outcome of infertility treatment in older patients. *Fertil Steril*. 2010;93(1):272–5.
2. Munné S, Sandalinas M, Magli C, Gianaroli L, Cohen J, Warburton D. Increased rate of aneuploid embryos in young women with previous aneuploid conceptions. *Prenat Diagn*. 2004;24(8):638–43.
3. Fragouli E, Escalona A, Gutiérrez-Mateo C, Tormasi S, Alfarawati S, Sepulveda S, et al. Comparative genomic hybridization of oocytes and first polar bodies from young donors. *Reprod Biomed Online*. 2009;19(2):228–37.
4. Rodríguez-González M, Serra V, García-Velasco JA, Pellicer A, Remohí J. The “vanishing embryo” phenomenon in an oocyte donation programme. *Hum Reprod*. 2002;17(3):798–802.
5. World Health Organization. WHO laboratory manual for the examination of human sperm and semen-cervical mucus interaction. 4th ed. New York: Cambridge University Press; 1999.
6. Casper RF, Meriano JS, Jarvi KA, Cowan L, Lucato ML. The hypo-osmotic swelling test for selection of viable sperm for intracytoplasmic sperm injection in men with complete asthenozoospermia. *Fertil Steril*. 1996;65(5):972–6.
7. Jurisicova A, Varmuza S, Casper RF. Programmed cell death and human embryo fragmentation. *Mol Hum Reprod*. 1996;2:93–8.
8. Ziebe S, Lundin K, Loft A, Bergh C, Nyboe Andersen A, Selleskog U, et al. FISH analysis for chromosomes 13, 16, 18, 21, 22, X and Y in all blastomeres of IVF pre-embryos from 144 randomly selected donated human oocytes and impact on pre-embryo morphology. *Hum Reprod*. 2003;18:2575–81.
9. Wilding M, Di Matteo L, Dale B. The maternal age effect: a hypothesis based on oxidative phosphorylation. *Zygote*. 2005;13(4):317–23.
10. Bartmann AK, Romão GS, Ramos Eda S, Ferriani RA. Why do older women have poor implantation rates? A possible role of the mitochondria. *J Assist Reprod Genet*. 2004;21(3):79–83.

11. Simón C, Cano F, Valbueña D, Remohí J, Pellicer A. Clinical evidence for a detrimental effect on uterine receptivity of high serum oestradiol concentrations in high and normal responder patients. *Hum Reprod.* 1995;10:2432–7.
12. Valbuena D, Martin J, de Pablo JL, Remohí J, Pellicer A, Simón C. Increasing levels of estradiol are deleterious to embryonic implantation because they directly affect the embryo. *Fertil Steril.* 2001;76:962–8.
13. Chun SY, Hsueh AJ. Paracrine mechanisms of ovarian follicle apoptosis. *J Reprod Immunol.* 1998;39(1–2):63–75.
14. Nelson JR, Feinman M, Wilcox J, Batzofin J. Preimplantation genetic diagnosis in embryos created from oocyte donation. *Fertil Steril* 2001 pp. S76 ASRM abstract.
15. Munné S, Ary J, Zouves C, Escudero T, Barnes F, Cinioglu C, et al. Wide range of chromosome abnormalities in the embryos of young egg donors. *Reprod Biomed Online.* 2006;12(3):340–6.