Paternal age and assisted reproductive technology outcome in ovum recipients

This study suggests that paternal age may be inversely associated with reproductive outcome, as demonstrated by a decline in fertilization, blastocyst formation, implantation and cryopreservation rates with advancing age. (Fertil Steril® 2009;92:1772–5. ©2009 by American Society for Reproductive Medicine.)

An increasing trend in delaying childbirth has been noted in industrialized countries. This change is reflective of the changing role of women in society no longer solely ruling the sphere of domesticity, but pursuing careers in wide ranging fields. Although the influence of advancing maternal age on infertility has been well studied and documented, the effect of paternal age on infertility has been the subject of few investigations (1).

Although studies have demonstrated that aging women have increasing aneuploidy rates due to meiotic errors, controversy exists as to whether embryonic development is influenced by the age of the male partner. Multiple investigators have concluded that advancing paternal age negatively impacts reproductive and IVF outcome (2–7). A decrease in serum steroid levels has been documented in older men and controversial results have been reported about the effects of aging on sperm motility, morphology, and concentration (8–11). A review of the literature suggests a detrimental effect on semen volume, sperm motility and morphology, particularly in men >50 years (12). With respect to the clinical reproductive outcome of older men, the data in most studies are difficult to interpret due to the lack of adjustment for female age. To reduce the profound effect that the aging women (oocyte) has on reproductive potential, and because older men tend to reproduce with older women, we decided to evaluate the effect of paternal age on embryo development and IVF outcome in ovum donation (OD) cycles at our center. By only using OD cycles we controlled for the effects of female aging and declining fertility, making this an analysis of the males' effect on the embryo and cycle outcomes.

Martha Luna, M.D.
Elissa Finkler, B.S.
Jason Barritt, Ph.D.
Natan Bar-Chama, M.D.
Benjamin Sandler, M.D.
Alan B. Copperman, M.D.
Lawrence Grunfeld, M.D.
Mount Sinai School of Medicine, Department of Obstetrics and Gynecology, Department of Reproductive Endocrinology and Infertility and Reproductive Medicine Associates of New York, New York, New York

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Reprint requests: Alan B. Copperman, M.D., Department of Obstetrics and Gynecology, Division of Reproductive Medecine, 635 Madison Ave, 10th Floor, New York, NY 10022 (FAX: 212-756-5770; E-mail: acopperman@rmany.com).

We retrospectively studied all fresh OD cycles from January 2003 to December 2007 at Reproductive Medicine Associates of New York. Only cycles in which donors were <35 years with normal baseline ovarian reserve testing (FSH <10 IU/L and basal antral follicle count >8) were analyzed. Cases using donor sperm, testicular sperm, or aspirated sperm were excluded from analysis. Cycles were classified by male partner's age: group A <40 years; group B 40–49 years; group C >50 years. Variables analyzed within each group included: total motile sperm in fresh ejaculate the day of the retrieval, fertilization (2PN) rate, number of day 3 embryos with ≥7 cells, blastocyst formation rate, day of embryo transfer, mean number of embryos transferred, number of cryopreserved embryos, cancellation, implantation, clinical pregnancy (PR), and pregnancy loss rates, along with female recipient's age and endometrial thickness.

Oocyte donors and recipients completed a standard screening protocol based on the American Society for Reproductive Medicine (ASRM) guidelines (13), including documentation, in the recipient, of a normal uterine cavity and adequate endometrial development with a preparatory cycle before embryo transfer. Donor cycles are offered to recipients as shared or nonshared. A shared cycle implies that a primary and secondary recipient will have the same donor's oocytes randomly split between the two after retrieval, with 12 being the minimum number, to proceed with a shared cycle. If less than 12 oocytes are retrieved, they all are assigned to the primary recipient.

Fresh ejaculated semen samples are evaluated the day of the oocyte retrieval by standard andrological screening techniques. In general, raw semen specimens with <20 million total motile sperm are considered inadequate for conventional insemination (CI) and therefore are prepared for use with intracytoplasmic sperm injection (ICSI). The CI is performed 4–6 hours after retrieval by mixing one to three oocyte/cumulus complexes in 50-μL media drops with 50,000 motile sperm for 16–18 hours. For ICSI, oocytes are denuded of cumulus–coronal cells 3–4 hours after retrieval by exposure to hyaluronidase. Mature oocytes after hyaluronidase exposure are then injected with a single sperm 5–6 hours after retrieval. Fertilization is confirmed 16–18 hours after CI or ICSI. Embryos are assessed daily until the day of embryo transfer or day of cryopreservation.

At our center, embryo transfers are performed under transabdominal ultrasound guidance using a Wallace catheter (Marlow Technologies, Willoughby, OH) with cleavage or blastocyst-stage embryos. Our laboratory policy on transfer of embryos derived from donor oocytes is to transfer two embryos, either on day 3 or day 5, based on morphology. The blastocyst formation rate is

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TABLE 1

Semen parameters and cycle outcome by male age groups.

Outcome by male age	< 40 years (n = 233)	40–49 years (n = 323)	> 50 years (n = 116)
Male age (y)	35.5 ± 2.9	44.1 ± 2.9	54.3 ± 4.5
Semen parameters			
Volume (mL)	3.67 ± 8.5[a]	2.7 ± 1.5[a,b]	2.1 ± 1.5[a,b]
Concentration (million/mL)	75.5 ± 54.6[a]	76.2 ± 62.7[b]	58.6 ± 56.5[a,b]
Motility (%)	56.8 ± 14.2[a]	52.8 ± 16.1[a,b]	42.9 ± 18.3[a,b]
Total motile (million)	261.6 ± 116.2[a]	159.9 ± 162.5[a,b]	110.0 ± 151.9[a,b]
Cycle outcome			
Oocyte age (y)	26.2 ± 3.2	25.9 ± 3.1	26.6 ± 3.4
No. of oocytes/recipient	13.1 ± 6.8	13.7 ± 7.1	12.4 ± 5.4
Cycles with ICSI (%, n)	33.0%[a] (77)	38.1%[b] (123)	65.5%[a,b] (76)
Fertilization rate (2PN/oocytes retrieved)	63.7%[a] (1,951/3,062)	60.3%[a] (2,663/4,414)	58.8%[a] (845/1,437))
Fertilization rate CI/ICSI	63.9%/63.5%	61.6%/59%	54.9%[a]/60.6%[a]
Day 3 embryos with >7 cells (embryos/2PN)	66.2%[a] (1,292/1,951)	63.1%[a] (1,679/2,663)	61.5%[a] (520/845)
Blastocyst formation rate (only blastocyst embryo transfer)	61%[a] (658/1,078)	51.6%[a] (811/1,573)	47.8%[a] (185/387)
Day 5 embryo transfer (%)	60.4% (136/225)	58.6% (184/314)	50.4% (58/115)
No. of embryos transferred (day 3/day 5)	2.2 ± 0.8 (2.2/2.1)	2.2 ± 0.7 (2.3/2.1)	2.2 ± 0.6 (2.3/2.3)
Cancelled embryo transfer	8/233 (3.4%)	9/323 (2.8%)	1/116 (0.8%)
Cryopreserved embryos day 6 (%)	27.4%[a] (534/1,951)	20.5%[a] (546/2,663)	15.4%[a] (130/845)
Cryopreserved embryos/recipient	2.3 ± 3.5[a]	1.7 ± 3.4[a]	1.1 ± 1.7[a]
Implantation rate	44.8% (229/511)	44.8% (321/716)	40% (104/260)
Clinical pregnancy rate	61.4% (143/223)	63.2% (204/323)	69% (80/116)
Loss rate	11.9% (17/143)	15.2% (31/204)	15% (12/80)
Endometrial thickness (mm)	9.3 ± 2.5	9.5 ± 2.4	9.4 ± 2.2
Recipient age (y)	39.3 ± 5.1[a]	43.7 ± 3.0[a]	45.5 ± 3[a]

CI = conventional insemination; ICSI = intracytoplasmic sperm injection; PN = pronuclei.
[a] $P<.05$.
[b] $P<.05$.

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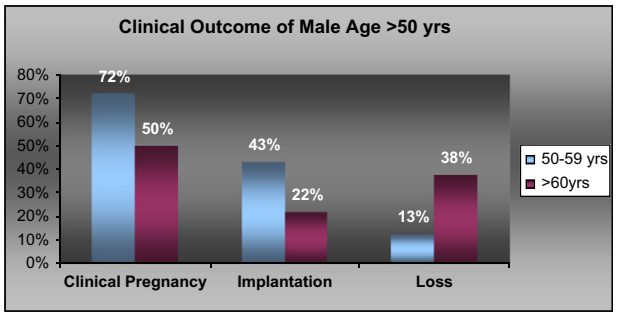
determined by the number of fertilized oocytes that develop into blastocysts. This rate was calculated only for cases that underwent a day 5 embryo transfer, eliminating from analysis, those cases that had a day 3 embryo transfer. After embryo transfer, surplus embryos are maintained in culture and reassessed on day 6. Cryopreservation is reserved for fully expanded embryos with an inner cell mass (ICM) and trophectoderm of A or B (Gardner classification). A pregnancy test is performed 16 days after hCG trigger. The first ultrasound to confirm the presence of an intrauterine pregnancy is performed 18 days after embryo transfer. A clinical pregnancy is defined by the presence of an intrauterine gestational sac. A loss is defined by the lack of embryonic growth or development after visualizing a gestational sac through transvaginal ultrasound (TVS).

The study included 672 OD cycles and were classified as follows: group A, 233 men <40 years; group B, 323 men 40–49 years; and group C, 116 men >50 years. According to semen parameters, the volume, sperm concentration, and total motile sperm count were significantly lower with increasing age, requiring the utilization of ICSI significantly more often in older age groups. The mean oocyte age and number of assigned oocytes were similar among groups. Overall, fertilization rates significantly decreased with advancing age; however, when categorized by type of insemination (ICSI vs. CI), these rates were not different for age groups A and B, but were noted to be significantly lower for those cases that had undergone CI in group C. The number of embryos with ≥7 cells on day 3 and the blastocyst formation rate significantly decreased as partner's age progressed ($P<.05$). Although, the embryo stage at transfer and the mean number of transferred embryos were not different, the number of embryos available to cryopreserve on day 6 significantly decreased with advancing age ($P<.05$). Clinical PR, implantation, and loss rates were maintained in the three age groups (Table 1). When men in group C were subdivided by age (50–59 years and >60 years), a significant decline in implantation rates ($P=.022$) was noted in the latter. A trend toward a lower clinical PR (72% [72/100] vs. 50% [8/16]; $P=.14$) and a higher loss rate (13% [9/72] vs. 38% [3/8]; $P=.17$) was also noted for men >60 years (Figure 1). The mean thickness of the recipient's endometrium was analyzed and did not vary between groups.

It has been well documented that as women get older, there is an increased risk of infertility. However, attention is now being turned to the effects of paternal age on infertility and fecundity. We conducted a study including only fresh IVF cycles using donor oocytes. In our study, in addition to evaluating outcome rates, we evaluated embryo development. Although a subtle, inverse association between male age with fertilization and blastocyst formation is seen, our results demonstrate a strong, negative correlation between paternal age >60 years and reproductive outcome, specifically for implantation.

FIGURE 1

Cycle outcome of male partner's age: 50–59 and >60 years. A significant decline ($P=.022$) was noted for implantation rates in male age group >60 years. A trend toward a lower clinical pregnancy rate (PR) (72% [72/100] vs. 50% [8/16]; $P=.14$) and a higher loss rate (13% [9/72] vs. 38% [3/8]; $P=.17$) was also noted for men >60 years.



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In summary, our results demonstrate a significant decline in fertilization rates with advancing paternal age. When comparing this variable by insemination technique, we found that CI was associated to lower a fertilization yield only for male partners older than 50 years, regardless of having optimal semen parameters. Despite these findings, clinical outcome parameters were not different between groups A, B, and C. However, upon subcategorizing group C, implantation rates were noted to be significantly lower for men >60 years. A trend toward lower clinical PRs and higher loss rates was also documented for this age group, despite having similar semen parameters. Although the female partners from group C were significantly older when compared with groups A and B, their ages were not different after subdivision of men into 50–59 years and >60 years. It is relevant to point out that the patient population in this subgroup was limited in number. The inverse association between paternal age and fertilization rate, blastocyst formation rate, implantation rate, and the mean number of available embryos to cryopreserve in men older than 60 years, may result from genetic mutations that accumulate in the spermatagonial stem cells during continuous cell division and may lead to abnormal sperm production.

We demonstrated that later embryo development, after the cleavage stage, may be significantly affected by aging sperm leading to a significant decrease in blastocyst formation rate, which is contemporaneous with the male genomic activation within the embryo. Earlier cytogenetic findings have implied an inverse paternal age effect on the overall incidence of numerical chromosomal aberrations, and recent studies with fluorescence in situ hybridization suggest that sperm of men of about 40 years contain more aneuploid cells than those of 20-year olds (14). An increased number of mutations in the sperm of older fathers may have subsequently increased adverse embryo development and IVF outcome. This is consistent with our hypothesis that an increase in abnormal sperm production occurs with advancing paternal age. However, this increase in abnormal sperm production may have only a minor effect on final fertility outcome as demonstrated by our results.

Because our study only evaluated donor eggs in women <35 years the female contribution to aneuploidy was minimized. Although donors in their early 20s yield higher PRs than those in their 30s, the mean donor's age was constant in all our groups. The consistent endometrial preparation between groups excluded an endometrial receptivity factor. Endometrial thickness was within normal parameters as to not affect fertility. The Center for Disease Control and Prevention report of 2006 does not demonstrate a different live birth rate in women <48 years of age when donor eggs are used (15). Zenke and Chetkowski (16) studied the recipient-related determinants of outcome with donor eggs for 134 embryo transfers. The study compared the women who achieved pregnancy and those who did not and found that an average endometrial thickness of 10.8 mm in the pregnant group was not statistically different from a thickness of 9.8 mm in the nonpregnant group. However, it was discovered that none of the pregnant patients had an endometrium <8 mm. Thus, a thin endometrium was one of the most important recipient-related determinants of success with donor eggs and success of achieving pregnancy may be reduced. Our results showed an average endometrial thickness of 9.3–9.8 mm across maternal age groups.

To our knowledge, four other studies have evaluated the impact of male age using an ovum donation model; however, the results have been contradictory (3, 17–19). Our results are consistent with those described by Frattarelli et al. (18), in that paternal age may have an impact on pregnancy outcomes and blastocyst formation rates. They conclude that male age >50 years significantly affects pregnancy outcomes and blastocyst formation rates. In our study, a significant decrease was noted in the blastocyst formation rate for the aging male. In addition, a significant decrease in implantation rate was noted only in pregnancies of male partner's older than 60 years. The critical age threshold with respect to sperm production is unknown; however, many studies have demonstrated that advanced paternal age is associated with diminished semen quality, therefore resulting in greater risk for infertility. Further prospective studies are needed to investigate the relationship between the degradation of DNA and reproductive outcome in male partner's older than 60 years of age. If further studies confirm that advancing paternal age is correlated with increased aneuploidy, gene mutations, and DNA damage and chromatin integrity, the clinician must revise current counseling procedures to ensure that patients are cognizant of such risks.

REFERENCES

1. Navot D, Bergh P, Williams A, Garrisi G, Guzman I, Sandler B, et al. Poor oocyte quality rather than implantation failure as a cause of age related decline in female fertility. Lancet 1991;337:1375–7.
2. Johnson L, Zane R, Petty C, Neaves W. Quantification of the human Sertoli cell population: its distribution, relation to germ cell numbers, and age related decline. Biol Reprod 1984;31:785.
3. de La Rochebrochard E, de Mouzon J, Thépot F, Thonneau P. Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. Fertil Steril 2006;85:1420–4.
4. de La Rochebrochard E, Thonneau P. Paternal age ≥ 40 years: an important risk factor for infertility. Am J Obstet Gynecol 2003;189:901–5.
5. Neme R, Ravizzini P, Carizza C, Abdelmassih S, Abdelmassih V, Abdelmassih R. Paternal age negatively influences embryo quality but not pregnancy

results in couples treated with intracytoplamatic sperm injections (ICSI). Fertil Steril 2007;88(Suppl 1):S373.
6. de La Rochebrochard E, Thonneau P. Paternal age: are the risks of infecundity and miscarriage higher when the man in aged 40 years or over? Rev Epidemiol Sante Publique 2005;53:S47–55.
7. Ford W, North K, Taylor H, Farrow A, Hull H, Golding J. Increasing paternal age is associated with delayed conception in a large population of fertile couples: evidence for declining fecundity in older men: the ALSPAC Study Team (Avon Longitudinal Study of Pregnancy and Childhood). Hum Reprod 2000;15:1703–8.
8. Schwartz D, Mayaux MJ, Spira A, Moscato ML, Jouannet P, Czyglik F, et al. Semen characteristics as a function of age in 833 fertile men. Fertil Steril 1983;39:530–5.
9. Gallardo E, Simon C, Levy M, Guanes P, Remohi J, Pellicer A. Effect of age on sperm fertility potential: oocyte donation as a model. Fertil Steril 1996;66: 260–4.
10. Neaves W, Johnson L, Porter J, Parker C, Pretty C. Leydig cell numbers, daily sperm production, and serum gonadotropin levels in aging men. J Clin Endocrinol Metab 1984;59:756–63.
11. Deslypere J, Kaufman J, Vermeulen T, Vogelaers D, Vandalem J, Vermeulen A. influence of age on pulsatile luteinizing hormone release and responsiveness of the gonadotrophs to sex hormone feedback in men. J Clin Endocrinol Metab 1987;64:68–73.
12. Kidd S, Eskenazi B, Wyrobek A. Effects of male age on semen quality and fertility: a review of the literature. Fertil Steril 2001;75:237–48.
13. American Society for Reproductive Medicine. Guidelines for oocyte donation. Fertil Steril 2002;77(Suppl 5):S6–8.
14. Eichenlaub-Ritter U. Paternal age-related aneuploidy in human germ cells and offspring: a story of past and present. Environ Mol Mutagen 1996;28:211–36.
15. Center for Disease Control and Prevention. 2006 Assisted Reproductive Technologies (ART) Success Rates - National Summary and Fertility Clinics Report: Section 4—ART cycles Using Donor Eggs. CDC and Prevention, Atlanta, GA; ASRM, Birmingham, Alabama. November, 2008.
16. Zenke U, Chetkowski R. Transfer and uterine factors are the major recipient-related determinants of success with donor eggs. Fertil Steril 2004;2: 850–6.
17. Paulson RJ, Milligan RC, Sokol RZ. The lack of influence of age on male infertility. Am J Obstet Gynecol 2001;184:818–22.
18. Frattarelli J, Miller K, Miller B, Elkind-Hirsch K, Scott R. Male age negatively impacts embryo development and reproductive outcome in donor oocyte ART cycles. Fertil Steril 2008;90:97–103.
19. Bellver J. Influence of paternal age on assisted reproduction outcome. Reprod Biomed Online 2008;17:595–604.