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Contribution of male age to outcomes in assisted reproductive technologies—addressing methodological challenges

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Abstract

Objective—To evaluate the relation between male age and pregnancy outcome in donor oocyte assisted reproductive technology cycles

Design—Retrospective cohort

Setting—Private IVF center.

Patients—1392 donor cycles from 1083 female recipients and their male partners

Interventions—Oocyte donor cycles

Main outcome measure(s)—Live birth

Results—Increasing male age was associated with semen parameters including volume and motility; however, male age was not observed to have a statistically significant association with likelihood of live birth in donor cycles after adjustment for female recipient age.

Conclusions—When treatment cycle number and female recipient age were taken into account, male age had no significant association with pregnancy outcomes in ART donor cycles in this study population.

Keywords

Male age; In vitro fertilization; Donor oocyte; Live birth rates

Introduction

The contribution of advancing female age to pregnancy outcomes is well recognized (1,2); however, that of male age is less well known. The mechanisms through which advancing male age affects outcomes are uncertain, but there are important implications of the

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association between age and outcomes in the context of assisted reproductive technologies (ART). The observation of higher rates of infertility and adverse outcomes—including early pregnancy loss and miscarriage—with increasing maternal age has been connected to a declining population of oocytes (3). Moreover, the oocyte population among older reproductive aged women is suggested to be of lower quality, and with a greater proportion of chromosomal abnormality in comparison with younger women (4). In contrast, little is known regarding the role of paternal age on pregnancy outcomes.

Anecdotal evidence suggests a substantially smaller role for effects of male aging in comparison with those of maternal age. Normal pregnancies of young women partnered with older males indicate the absence of a firm ceiling on male age in relation to reproductive potential. Prior research has evaluated the relation of semen characteristics with increasing male age. An association between male age and semen volume, motility and morphology has been observed (5,6); less consistent results have been observed for relations with pregnancy outcomes (7-11). Some investigators have suggested that the age of female recipients in donor cycles is unrelated to pregnancy outcomes (12). Nevertheless, the correlation between maternal age and adverse pregnancy outcomes may be related to endometrial aging or uterine factors that are related to age, such as fibroids (13). In unassisted pregnancy or in autologous embryo transfer, ovarian aging and aging of other reproductive factors are inseparable, leading to uncertainty regarding the roles of the embryo and its uterine environment on outcomes. For social reasons, maternal age is highly correlated with paternal age, and as a result, the relative contributions of maternal age and paternal age on pregnancy outcomes are challenging to separate (6). Statistical control for maternal age is one approach to make this assessment, but distinguishing the effects of paternal age from those of maternal age requires substantial sample sizes (14,15).

The oocyte donor model offers an opportunity to address many of the above challenges. Because donors are of similar ages and fertility, oocyte age-related factors are controlled by restriction, and the contribution of paternal age may be separable from effects the embryo environment (16). Because donor ages are uncorrelated with the ages of recipients and their male partners, the effects of oocyte age and recipient age may be separated. Through use of statistical adjustment, and given sufficient sample size, the relative contributions of recipient and male partner age may also be assessed. Nevertheless, an additional complication arises from the relation between patient age—both female and male—and the number of treatment cycles. Patients undergoing their first treatment cycle are younger than patients in subsequent cycles. As those who seek subsequent treatment cycles most frequently are doing so after a failed first cycle, cycle number is in turn related to pregnancy outcomes. Thus, observed associations between age and outcomes may be complicated by this selection factor.

Few prior studies have reported on paternal age using the donor egg model, and those that have report conflicting results. Paulson and colleagues observed no relation between paternal age and pregnancy outcomes; however, the size of the study was small and may have limited the ability to find associations (7). In a recent larger study by Frattarelli and colleagues, a statistically significant association was observed, however this analysis did not control for the effects of maternal recipient age (8). For this study, we sought to evaluate the independent contributions of recipient and paternal age to pregnancy outcomes while accounting for the treatment cycle number.

Methods

Study population and assessment of participant characteristics

Institutional review board approval was obtained. Anonymous oocyte donors were between 21-33 years old with a BMI ≤ 28 kg/m². A complete medical history including an assessment of relevant communicable diseases was obtained from all potential donors. Potential donors identified as being at high risk for infectious disease were disqualified. Genetic risks were established by evaluating a three-generation medical and genetic history and applicable genetic testing included cystic fibrosis carrier screening of all oocyte donors. Endocrine evaluation included a baseline day 3 estradiol, FSH, LH and TSH. If the baseline testing was normal, a urine drug screen and all FDA required sexually transmitted disease screening were obtained. Any reactive or positive testing resulted in donor disqualification. Donors underwent a thorough psychological evaluation to evaluate her readiness to undergo oocyte donation. A physical examination was performed including a baseline transvaginal ultrasound. After the donor was deemed eligible for donation and the FDA required 30-day screening of communicable diseases was satisfied, the donor was cleared to proceed with oocyte donation.

Screening of oocyte donation recipients included a mock exogenous estrogen cycle using a 2-week trial estrogen cycle followed by a mid-cycle endometrial thickness assessment with a target of ≥ 8 mm. Additional screening requirements included a hysterosalpingogram within the last 2 years and a mock embryo transfer with a sonohysterogram. Infectious disease testing of both the male and female partners was obtained. Additional testing included a TSH, PRL, Varicella, Rubella, CBC and PAP smear. If the recipient was ≥ 40 years old, a mammogram was obtained and if the recipient was ≥ 45 years old, an EKG and a fasting glucose were also obtained. A counseling session with a social worker or psychologist was required for all third-party cycles. Embryo transfers occurred at Shady Grove Fertility Reproductive Science Center between January of 2002 and January of 2006.

Factors evaluated included male age, semen analysis parameters (concentration, volume, percent motile), recipient age, ICSI use (yes/no), cycle number, and number of embryos transferred.

Outcome assessment

The primary outcome was live births. Additional variables analyzed included implantation and clinical pregnancy rates and spontaneous abortion. Determination of these outcomes was according to previously described methods (17). Briefly, if an appropriately rising serum hCG was encountered, a transvaginal ultrasound was performed at 6-7 weeks gestation to determine the presence of any gestational sacs. Clinical pregnancies were determined by the presence of a gestational sac on transvaginal ultrasound. The implantation rate was the number of gestational sacs per number of embryos transferred. The live birth and spontaneous abortion rates were defined as the proportion of transfers resulting in the respective outcomes.

Statistical analysis

The number of treatment cycles contributed by each of the 1083 couples—donor egg recipient and male partner—in the study population was assessed initially. Among the couples, the relation between recipient age and male partner age was crudely evaluated by Pearson correlation models. Mean recipient and paternal ages were compared by treatment cycle number using ANOVA models, and pair-wise group differences evaluated by Tukey *post hoc* tests.

Characteristics of the 1392 treatment cycles contributed by the 1083 recipient – male partner pairings were evaluated and compared between those with live births and those without. Because of the non-independence in the dataset resulting from repeat treatment cycles, comparisons of characteristics were evaluated using general linear models for correlated data for both continuous factors and for categorical factors. Nonparametric tests for continuous factors were also evaluated to address the potential effects of violation of distributional assumptions. Relations between patient characteristics and clinical parameters and measures of semen quality were also evaluated.

The relations between patient ages and pregnancy outcomes were assessed using multivariable techniques. Age was initially explored as a continuous variable; however, such models assume that the effect of a unit increase is the same across the range of age (e.g., equivalence in comparisons of 36 vs. 35 years of age and 46 vs. 45 years of age). Accordingly, age of recipients and their male partners was categorized to allow for departures from linearity and ease of interpretation of results. In order to evaluate the potential effects of treatment cycle number, these modeling approaches were applied in three different contexts: 1) to the whole dataset of 1398 treatment cycles with up to 6 treatment cycles per participant; 2) only among first treatment cycles ($n=1083$), and; 3) only among the most recent treatment cycles ($n=1083$) of the study participants. Likelihood of live births was assessed with generalized estimating equations (GEE) to address dependencies in the data with all 1392 observations, and using logistic regression models where only first or most recent treatment cycles were considered. Unadjusted models were evaluated initially; multivariable logistic regression models were constructed that included the primary factors of interest, recipient and paternal age.

Results

Table 1 shows the overall characteristics of the patients at their first treatment cycle in study population ($n = 1083$). Paternal age was significantly associated with maternal age, with an increase in mean maternal age with increasing paternal age group. Increasing male age was associated with a significant decrease in semen motility and volume ($p<0.001$), but there was no significant association between paternal age and sperm concentration ($p=0.61$). In this bivariate analysis, paternal age was not significantly associated with number of oocytes retrieved, inseminated, or fertilized, nor was it associated with the number of embryos frozen, pregnancy, or live birth. Increasing paternal age was associated with both ICSI use ($p<0.01$) and the number of embryos transferred ($p=0.02$).

Patient characteristics were examined by cycle number (Table 2). While semen characteristics did not vary significantly by cycle number, the number of oocytes retrieved increased as cycle number increased ($p<0.001$), as did number of embryos transferred ($p<0.05$) and ICSI use ($p<0.05$; Table 3). Implantation and live births both decreased with increasing treatment cycle number. Implantation for first treatment cycles was approximately 67%, 62% for second treatment cycles, and 58% for third treatment cycles ($p = 0.18$). Percentage of live births decreased by cycle number, from 58.5% of treatments resulting in live birth in cycle 1 to 42.9% in cycle 3 ($p<0.01$). Spontaneous abortion risks were not significantly different between cycle numbers, but were lowest in cycle 1 at 8.7% and highest for cycle 3 at 10.2%.

Table 3 shows the live birth rates among male-female partner pairings for non-cancelled cycles by age group of both male and female partner. The shaded area represents the pairings with male and female partners of similar age (i.e., ± 1 age group). The highest live birth rate observed was 83% (24/30, 95% CI = 66%-94%), for pairings with females in the youngest age group and male partners in the 35-39 age group. The upper right corner shows

a single failed transfer with female age <35 and male age 50+. Apart from this combination, the lowest live birth rate observed was 42% (14/33, 95% CI = 26%-60%), for pairings with a female in the 40-44 age group and male partners in the <35 age group.

To formally test the relations illustrated in Table 3, we evaluated the association between live birth likelihood and male age adjusting for female recipient age using logistic regression, and looking exclusively at first cycles (Table 4). In models of male age unadjusted for female age, we found statistically non-significant increased odds of live birth among the younger age groups in comparison with the male age 50+ reference category (OR range: 1.10 for men 40 - 44; 1.32 for men 35 - 39). After adjusting for the effects of maternal recipient age, results remained non-significant and point estimates were attenuated; odds ratio estimates were closer to one, and were less than 1 for men in the youngest age group. The test for trend was non-significant ($p=0.98$) and in no single age group was the association with live birth statistically significant. Analysis of live birth likelihood by categories of female age suggested an association between increasing age and decreasing live birth rates that was little in adjusted analysis that simultaneously considered male and female age. The greatest likelihood of live birth was observed in the youngest age group, followed by women 35 - 39, and likelihood was similar between those in the 40 - 44 and 45+ age groups; p -values from tests for trend were 0.09 for unadjusted and 0.13 for male age-adjusted analyses

Discussion

Increasing male age has been shown to be associated with infertility(10), as well as decreased semen motility and volume (5,6). A limited body of work exists on the association between paternal age and pregnancy outcome using the donor egg model (7,8). Prior research presents conflicting results, with some suggesting increased paternal age associated with poorer pregnancy outcomes (8) and others suggesting no association (7). Importantly, these studies have not addressed the role of female recipient age in analysis. In our analysis that adjusted for female partner age, we found decreased semen volume and motility with increasing age, but we did not find an association between paternal age and likelihood of live birth.

Our results are similar to those of Paulson and colleagues (7), who performed a retrospective analysis of 558 donor cycles in 441 couples and found no association between paternal age and live birth rate ($p=0.84$). Male age was categorized into quartiles, though the age ranges for these quartiles were not reported. Similar to our findings, these authors observed an association between increasing male age and certain, but not all, parameters of the semen analysis. In the Paulson *et al.* study, analysis considered all cycles contributed by a couple, used logistic regression without accounting for correlation between cycles, and did not control for maternal recipient age. In our data, we found no statistically significant association between paternal age and likelihood of live birth, evaluating first ART cycle exclusively and controlling for maternal recipient age.

Frattarelli *et al.* evaluated embryo characteristics and treatment cycle outcomes in 1023 anonymous oocyte donation cycles (8). Using efficiency curves to identify a cut point for age, and unadjusted analysis of live birth rates, these investigators found a significant decrease in live birth rate with paternal age >50 years old, relative to age ≤ 50 ($p<0.01$), as well as a decrease in semen volume and total motility with increasing paternal age ($p<0.05$). While oocyte age was effectively controlled for by restricting the sample to women receiving donor eggs, the authors did not control for maternal recipient age. In our data, the average recipient age among couples with male partners >50 years of age was 44.4 years. We observed decreasing live birth rates with increasing male age; however, adjustment for

female recipient age greatly attenuated this association. Our large sample size may have allowed for an improved ability to detect a relation between increasing female recipient age and outcomes of donor cycles (6,12,18).

There are important methodological issues presented when examining the association between paternal age and outcomes in ART including, but not limited to: 1) consideration of ART cycle number in subject selection; 2) use of appropriate statistical techniques for analysis, and; 3) consideration of female age at initiation of ART treatment.

Couples participating in ART often engage in more than one ART cycle. Intuitively, it is clear that cycle number is related to patient age, as age increases between cycles. We have shown this relation in our data, and also shown the potential for artifactual association between age and various factors related to cycle number. Increasing age is associated with pregnancy outcome in part because patients engaging in more than one cycle do so after a failed first cycle. Patients participating in first ART cycles are expected to be both younger and may have different outcomes than patients in subsequent ART cycles, suggesting use of first cycles when evaluating associations between age and ART cycle outcome. If most recent treatment cycles are evaluated, the effect of age may be overestimated given the increase in age that occurs between cycles and the decrease in likelihood of live birth. If all cycles are considered, statistical methods to account for correlated data must be considered (19). If the number of treatment cycles couples experience is meaningful, as is the case in the current context, more complicated methods for handling multiple cycle data may be needed (20). Using only first cycles results in a loss of statistical power but avoids potential biases that result from analysis of a cohort that includes the most recent cycle or improperly using all cycles.

We observed significant differences in patient characteristics, treatment factors, and outcomes among treatment cycle numbers. This observation reflects a mix of factors, including sorting by receptivity to treatment and alteration in treatment by physicians in response to failed cycles. The lower implantation rates in 2nd and 3rd cycles in comparison with 1st cycles illustrates the sorting process, whereas the increasing use of ICSI with repeated treatment cycles exemplifies how physicians use information from failed cycles to alter treatment in later cycles. Multiple treatment cycles contributed by a couple are decidedly not independent. We utilized only first cycles for our analysis to avoid this complexity, though at the cost of statistical power. Since our data suggest that a male age effect is likely to be small if an effect exists, extremely large samples will be necessary to detect it in order to appropriately handle artifactual effects of treatment cycle number and female recipient age.

For our analysis, we considered various approaches to address issues related to cycle number. These approaches included evaluating the association between paternal age and pregnancy outcome in the first cycle exclusively, evaluating the most recent cycle, evaluating all cycles without consideration of correlation between cycles, and finally evaluating all cycles using general estimating equations to account for the correlation between cycle number. In the present analysis, we found little difference in effect estimates for paternal age regardless of the modeling approach employed. For maternal age, however, while estimates using first cycle only and GEE to evaluate all cycles were of the same general magnitude and in the same direction (OR range, first cycle: 0.29 – 0.55; OR range, GEE: 0.54-0.84) estimates using most recent cycle only were in the opposite direction for some age groups (OR range, most recent cycles: 0.81-1.46).

Maternal recipient age is an important selection factor, and infertility factors vary across women of different ages. Younger women seeking fertility treatment are likely to do so for

reasons such as premature ovarian failure, in comparison with women seeking fertility treatment at age ≥ 35 with infertility related to decreasing oocyte quantity and quality. This complicates consideration of female age in the context of assisted reproductive technologies. It is therefore important to consider maternal age in appropriately refined categories to allow for differential risk across strata of maternal age.

We note that although we had a large sample size for the current size, with data for 1083 couples undergoing ART, our statistical power was limited after adjustment for recipient age. There were few couples with the widest range in age in our dataset (i.e. male partner ≥ 50 and female partner < 35), making it difficult to separate male and female age effects for the purpose of making inferences. Overcoming this limitation is challenging; couples with discordant ages are rare regardless of study size, adding to the difficulty of identifying the relations among oocyte age, female recipient age, and paternal age with outcomes. We used several approaches to regression adjustment in our large dataset to address these issues.

In conclusion, we found limited suggestion of an association between paternal age and live births among patients treated with ART. These results suggest that some of the effect of male age on outcomes of donor cycles previously observed has been falsely ascribed to paternal contributions, and instead are due to treatment cycle number and/or recipient age. If such an association exists, it is a weak one. This study adds to the limited literature using the donor egg model suggesting a limited contribution of paternal age to the live birth rates of ART cycles. Additionally, this study adds important considerations about the methodological issues surrounding analyses of the contribution of maternal and paternal age to ART success rates.

Capsule

The authors evaluated the relation between male age and outcomes in a cohort of 1392 consecutive ART donor cycles. No association was observed between male age and live birth rates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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