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Full Length Research Paper

Effect of paternal age on aneuploidy rates in first trimester pregnancy loss

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A retrospective cohort analysis of patients undergoing IVF cycles at an academic IVF center was performed to test the hypothesis that male age may influence aneuploidy rates in first trimester pregnancy losses. All patients had a first trimester pregnancy loss followed by evacuation of the pregnancy and karyotyping of the abortus. Couples undergoing anonymous donor oocyte ART cycles (n = 50) and 23 couples with female age less than 30 years undergoing autologous oocyte ART cycles were included. The oocyte age was less than 30 in both groups; thereby allowing the focus to be on the reproductive potential of the aging male. The main outcome measure was the effect of paternal age on aneuploidy rate. No increase in aneuploidy rate was noted with increasing paternal age (<40 years = 25.0%; 40-50 years = 38.8%; >50 years = 25.0%). Although there was a significant difference in the male partner age between oocyte recipients and young patients using autologous oocytes (33.7 7.6 vs. 41.5 6.8) (p <0.0001), no difference in aneuploidy rate was observed between these groups (30.0% vs. 26.1%) (p=0.66; RR=0.93; 95% CI (0.68-1.27)). While controlling for female age by using donor oocyte patients, the study's preliminary data support that paternal age does not affect aneuploidy rates.

Key words: Male age, *in vitro* fertilization, donor oocyte, aneuploidy rates, cytogenetics.

INTRODUCTION

Maternal age has been closely linked in multiple studies to reproductive success. Increased maternal age results in decreased fertility rates and decreased embryo implantation rates in assisted reproductive technology cycles (Navot et al., 1991; Battaglia et al., 1996; Selvin et al., 1976; Benadiva et al., 1996) and is accompanied by increased rates of chromosomal anomalies as well as miscarriage (Angell, 1994; Munne et al., 1995). impact of of paternal age on reproductive outcome is less clearly defined. Increased paternal age has been linked to decreased fecundity and adverse pregnancy outcome in the general population (Ford et al., 2000) and has also been linked to spontaneous abortion in the general population, even when maternal age is been controlled (Kleinhaus et al., 2006).

Morphological and functional alterations in the aging testes coupled with hypothalamic-pituitary axis aging result in decreased serum steroid levels in aging males (Neaves et al., 1984; Deslypere et al., 1987; Johnson et al., 1984). Some reports have suggested a decline in motility and morphologic semen characteristics, while other reports have noted no change in semen characteristics (Schwartz et al., 1983; Gallardo et al., 1996; Johnson et al., 1984). Vagnini et al. (2007), which

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Abbreviations: IVF, *In vitro* fertilization; **ART,** assisted reproductive technology.

demonstrated an increase in sperm DNA damage with increasing male age over 35 years in infertile males. Tarozii et al. (2007) proposed that the analysis of sperm DNA integrity may be used as an independent measure of semen quality as it may offer more accurate information than the traditional parameters of semen evaluation. A review of studies evaluating the effects of male age on fertility and semen quality concluded that increasing age of the male partner is associated with lower semen volume, motility and morphology; however, no change in sperm concentration was noted (Kidd et al., 2001). This review found that male fertility declines somewhat with age, particularly in men older than 50 years. Another recent review concluded that the potential of aging males' spermatozoa to fertilize oocytes *in vitro* is not altered, but there exists a risk of structural chromosomal abnormalities and autosomal dominant genetic diseases in offspring (Plas et al., 2000).

A study that analyzed success rates in 558 IVF cycles, with donated oocytes, found no paternal age effect on fertilization or live birth rate (Paulson et al., 2001). Other studies evaluating IVF pregnancies have suggested a decrease in reproductive success as paternal age advances (Klonoff-Cohen et al., 2004; Legro et al., 1995; Frattarelli et al., 2008; Luna et al., 2009).

Clearly, there is no adrenopause in males that is comparable to female menopause. Multiple studies have demonstrated that maternal aging is closely related to meiotic abnormalities and thus aneuploidy (Benadiva et al., 1996; Angell, 1994; Munne et al., 1995). However, the question of whether a similar process exists in the male remains unanswered. Since older men tend to reproduce with older women, it is difficult to eliminate the influence that the aging woman has on reproductive potential of the male (Frattarelli et al., 2008; Luna et al., 2009). In a recent report, Aboulghar et al. (2007) found that paternal age over 50 years had no effect on pregnancy rates in patients undergoing ICSI. However, long-term outcomes of these pregnancies were not reported. Interestingly, younger men had better fertilization rates in this study.

In the clinical practice of oocyte donation, oocytes from a young homogeneous population of donors eliminate the effect of maternal age on reproductive outcome. A recent review concluded that IVF with the use of donor oocytes from young women is highly successful, and the rate of pregnancy appears to vary little according to the age of the recipient. The average live-birth rate per embryo transfer for donor oocyte cycles is approximately 50%, indicating that the reduced rate of pregnancy associated with aging is a direct result of diminished ovarian function and oocyte quality. Consequently, this is not due to a reduction in endometrial receptivity (Van Voorhis, 2007). Two recent studies have suggested that embryo implantation and pregnancy outcome after oocyte donation are independent of the age or diagnosis of the female (Legro et al., 1995; Paulson et al., 2001). Therefore, oocyte donation is an optimal model to study the influence of male aging on reproductive potential.

The purpose of this investigation was to determine if the increasing male age has an impact on aneuploidy rates. The donor oocyte model was used to control the maternal aging effect.

MATERIALS AND METHODS

Population

From a retrospective cohort analysis of all IVF cycles performed at Reproductive Medicine Associates of New Jersey from January 2000 to December 2006, 413 patients who had a first trimester pregnancy loss followed by a dilatation and curettage as well as a karyotype analysis of the abortus were identified. From this patient population, all couples (n = 50) who underwent donor oocyte ART cycles using anonymous young donors were identified. Additionally, all couples (n = 23) with the female age of less than 30 years undergoing autologous oocyte ART were identified. This group was chosen to approximate the mean age of the anonymous young donor group.

All ovum donors were ≤ 35 years at the time of participation and passed standard screening criteria as outlined by the American Society for Reproductive Medicine (2002). All donors had normal FSH concentrations (<12 mIU/mL), a basal antral follicle count >8 and a normal gonadotropin responsiveness with peak estradiol levels > 500 pg/mL. All recipients had endometrial thickness of ≥ 8 mm prior to embryo transfer and all sperm was obtained by ejaculation.

Experimental design

IRB approval was obtained from the Western Institutional Review Board, Olympia, WA. The oocyte donor model was chosen to control for oocyte quality, while evaluating male age effect on aneuploidy. All basal FSH measurements were made within 6 months of the initiation of the treatment cycle. The ovum donors and autologous cycle patients underwent stimulation with a variety of stimulation protocols including GnRH-a (Lupron; TAP Pharmaceuticals, Deerfield, IL) down regulation followed by a stimulation with exogenous gonadotropins or stimulation with exogenous gonadotropins followed by use of a GnRH antagonist. When ultrasonographic criteria for follicular maturity were met, a single 10,000 IU dose of human chorionic gonadotropin (hCG) or its equivalent was administered. Transvaginal follicular aspiration was performed approximately 36 h after hCG administration. Embryos were transferred from 72 - 120 h after follicular aspiration.

Documentation of normal uterine cavity anatomy was established by saline sonohysterography or hysteroscopy within 12 months prior to the initiation of the IVF cycle. All recipients completed a standard testing protocol based on the American Society for Reproductive Medicine guidelines in screening the potential ovum recipients. All recipients completed a preparatory hormone replacement cycle to document appropriate endometrial response before the actual treatment cycle as described previously http://gateway1.ovid.com/ (Younis et al., 1992; Michalas et al., 1996; Navot et al., 1986).

Recipients with limited but demonstrable ovarian function were given subcutaneous leuprolide acetate, 1 mg/day, starting in the mid-luteal phase of the preceding menstrual cycle. Leuprolide acetate was not prescribed for patients with a complete ovarian senescence. Appropriately suppressed serum E_2 and progesterone levels and the presence of an early proliferative endometrial pattern

on transvaginal ultrasonography were confirmed before sequentially increasing doses of oral micronized 17β-estradiol (Estrace; Mead Johnson, Evansville, IN) were given. Serial serum E_2 measurements and transvaginal sonographic evaluation of the endometrium were monitored as previously described (Navot et al., 1986). After adequate endometrial proliferation and serum E_2 concentration were documented, leuprolide acetate treatment was discontinued and treatment with intramuscular progesterone in oil, 50 mg/day, was initiated on the evening of donor oocyte retrieval.

Sperm preparation and fertilization

Fresh ejaculated samples were evaluated by standard andrological screening (volume, count, motility, progression and Kruger morphology) (Baker et al., 2000). Following this analysis, the semen samples were prepared for conventional or ICSI insemination by density gradient separation followed by a swim up purification step. Specimens with less than 2 million motile spermatozoa per ejaculate, and/or abnormal morphology greater than 95%, were considered insufficient for standard conventional *in vitro* fertilization and were treated with ICSI. As a result, no testicular biopsy or sperm aspiration procedures were included.

For conventional insemination, oocytes were exposed to aggressively motile sperm from 4 - 6 h post oocyte retrieval. For ICSI, oocytes were denuded of cumulus-coronal cells by exposure to hyaluronidase and gentle aspiration of oocytes with a finely pulled pipette to remove residual cumulus-coronal cells and were then injected from 6 - 8 h in post oocyte retrieval. Fertilization was confirmed in 16 - 18 h post insemination by the confirmation of two distinct pronulei and polar bodies and was placed into culture according to established laboratory protocol (Miller et al., 2004). Embryonic division and morphology was evaluated every 24 h thereafter, until embryos were deemed suitable for embryo transfer or cryopreservation.

Statistical analysis

Male partners were stratified into age categories at 10 year intervals to assess the primary outcome measure of aneuploidy rate. Data were initially categorized by male ages at 10 year increments starting at age 40 and the outcome rates were then calculated for each 10 year increment. Efficiency curves were used to define an age threshold at which outcome rates were affected. Contingency table analyses were then used to evaluate the outcome rates above and below the selected threshold value. Differences in outcome rates were analyzed using a Chi-square or two-tailed Fisher's exact test, where appropriate.

Data were analyzed by using a t-test for continuous distributions and a Mann-Whitney Rank Sum Test for non-continuous distributions. An alpha error of 0.05 was considered significant for all comparisons. All data are reported appropriately as means with their associated standard deviations or as medians with their associated standard error of the mean. Relative risk and 95% confidence intervals are shown where appropriate (JMP 7, SAS Statistical Discovery Software).

RESULTS

This study patient population consisted of 50 patients who had a documented singleton intrauterine pregnancy following an oocyte donation cycle. All the patients were subsequently diagnosed with a missed abortion and underwent a dilation and curettage. Cytogenetic results from products of conception were reviewed. Patients were grouped by cytogenetic results as either normal or abnormal. The mean age of the female patients was 39.6 ± 5.0 years (range: 27 - 48 years), while that of the male was 41.5 ± 6.8 years (range: 31 - 59 years) and that of the donor was 27.8 ± 3.9 years (range 21 - 35 years).

Patient demographics and semen parameter results of the specimen used for fertilization on the day of oocyte retrieval, as well as serum β-hCG levels on days 16 and 18 are shown in Table 1. There was no difference between both oocyte recipients with normal and abnormal cytogenetics. There was no change in sperm concentration, motility or morphology throughout the study groups.

Table 2 shows the autologous and donor cycle parameters. Basal antral follicle number, days of stimulation, ampules of gonadotropins used, peak estradiol levels, number of oocytes, number of matured oocytes, number of fertilized embryos, endometrial thickness on the day of HCG and follicles ≥ 14 mm on the day of hCG were similar for both oocyte recipients with normal and abnormal cytogenetics.

Aneuploidy was found in 30.0% of abortuses among patients undergoing oocyte donation. Figure 1 shows percent aneuploidy grouped by paternal age. No increase in fetal aneuploidy rate was noted with increasing paternal age (<40 years = 25.0%, 40 - 50 years = 38.8%, >50 years = 25.0%). There was no statistical significant difference in aneuploidy rate when comparing the rates of the men <40 years to the men ≥ 40 years (25.0 versus 34.6%) ($p = 0.46$; RR = 1.15; 95% CI 0.80 - 1.65). Likewise, there was no statistical significant difference in aneuploidy rate when comparing the rates of the men <50 years to the men ≥ 50 years ($p = 0.93$; RR = 0.92; 95% CI 0.59 - 1.44). No difference in aneuploidy rates were noted between patients undergoing conventional IVF (n = 27) and those undergoing ICSI (n = 23).

As a second part of the analysis, the cytogenetic results of the 50 oocyte recipient couples were compared to a group of 23 female patients younger than 30 who had a missed abortion following IVF using autologous oocytes during the same period. Thus, oocyte age was less than 30 years for both groups, thereby allowing the evaluation of paternal contribution to aneuploidy. Table 1 shows patient demographics and semen parameter results for the specimen used in fertilization on the day of oocyte retrieval, as well as β-hCG days 16 and 18 for the oocyte recipient and autologous oocyte patients.

The age of the female patients in oocyte recipient group was significantly greater (39.6 ± 5.0 years) than the autologous oocyte group (28.7 ± 1.1 years) and that of the male partner in the oocyte recipient group was significantly greater (41.5 ± 6.8 years) than the autologous oocyte group (33.7 ± 7.6 years) ($p < 0.001$) (Table 1). The oocyte age in the oocyte recipient group was not significantly different than the autologous oocyte group. There was no difference in sperm concentration, motility

Table 1. Patient demographics and semen parameters for the oocyte recipients and the young autologous oocyte patients. Data are also subdivided into the oocyte recipients with normal abortus cytogenetics and abnormal abortus cytogenetics.

	Oocyte recipients	Oocyte recipients with normal cytogenetics	Oocyte recipients with abnormal cytogenetics	†P	Autologous oocyte females younger than 30 years	‡P
N	50	35	15		23	
Female age	39.6 ± 5.0	39.3 ± 5.4	40.3 ± 4.4	0.53	28.7 ± 1.1	<0.0001
Male age	41.5 ± 6.8	41.1 ± 6.9	42.3 ± 6.5	0.56	33.7 ± 7.6	<0.0001
Oocyte age	27.8 ± 3.9	27.5 ± 3.6	28.7 ± 4.4	0.34	28.7 ± 1.1	0.10
Volume (mL)	1.6 ± 0.5	1.5 ± 0.5	1.6 ± 0.5	0.85	1.5 ± 0.7	0.75
Concentration (mil/mL)	16.0 ± 20.8	11.9 ± 14.4	25.9 ± 31.6	0.27	10.8 ± 16.6	0.08
Morphology (%)	5.8 ± 4.2	6.0 ± 4.6	5.6 ± 3.1	0.81	4.7 ± 4.7	0.14
Motility (%)	92.6 ± 14.5	88.1 ± 19.9	91.6 ± 8.2	0.24	73.3 ± 36.1	0.18
*Number of embryos transferred	2.0 ± 0.1	2.0 ± 0.1	3.0 ± 0.2	0.83	2.0 ± 0.1	0.26
β-hCG day 16	127 ± 96	125 ± 90	134 ± 108	0.32	121 ± 78	0.81
β-hCG day 18	294 ± 237	282 ± 209	317 ± 279	0.28	284 ± 208	0.85

All values are depicted as means ± SD or *medians ± SEM as appropriate.† Mann-Whitney Rank Sum Test for non-continuous distributions or t-test for continuous distribution when comparing oocyte recipients with normal cytogenetic results to those with abnormal cytogenetic results. ‡Mann-Whitney Rank Sum Test for non-continuous distributions or t-test for continuous distribution when comparing oocyte recipients to autologous oocyte females younger than 30 years.

Table 2. Cycle stimulation parameters for oocyte donors and the young autologous oocyte patients. Data are also subdivided into the oocyte donors with normal abortus cytogenetics and abnormal abortus cytogenetics.

	Oocyte donor cycles	Oocyte donor cycles with normal cytogenetics	Oocyte donor cycles with abnormal cytogenetics	†P	Autologous oocyte females younger than 30 years	‡P
N	50	35	15		23	
Basal antral follicle number	10.7 ± 8.0	10.1 ± 7.7	11.7 ± 8.3	0.34	18.0 ± 10.8	<0.01
*Days of stimulation	10.1 ± 0.5	9.9 ± 0.3	10.0 ± 0.8	0.91	9.4 ± 0.7	0.63
Total ampules of gonadotropins	41.9 ± 15.1	40.2 ± 15.0	46.8 ± 14.4	0.15	37.7 ± 17.5	0.37
*Oocyte number retrieved	12.0 ± 1.8	12.0 ± 1.9	11.0 ± 3.2	0.77	18.0 ± 1.9	0.26
*Number of fertilized embryos	12.0 ± 1.2	7.0 ± 1.1	10.0 ± 2.2	0.17	8.0 ± 1.4	0.34
* ≥ 7-cell development day 3	3.0 ± 0.6	3.0 ± 0.7	3.0 ± 0.3	0.75	4.0 ± 1.1	0.13
Peak estradiol	1557 ± 869	1710 ± 970	1418 ± 744	0.85	2594 ± 1297	<0.001
* Follicles ≥ 14 mm on the day of hCG	12.0 ± 1.3	13.0 ± 1.2	11.0 ± 1.6	0.27	17.0 ± 2.1	0.22
Endometrial thickness on day of hCG	9.7 ± 2.2	9.3 ± 2.0	10.6 ± 2.2	0.83	9.9 ± 2.4	0.48

All values are depicted as means ± SD or *medians ± SEM as appropriate. † Mann-Whitney Rank Sum Test for non-continuous distributions or t-test for continuous distribution when comparing the oocyte donor cycles with normal cytogenetic results to those with abnormal cytogenetic results. ‡Mann-Whitney Rank Sum Test for non-continuous distributions or t-test for continuous distribution when comparing the oocyte donor cycles to autologous oocyte cycles in a group of females younger than 30 years.

motility, morphology or β-hCG on days 16 and 18 between these two groups.

Table 2 reveals cycle parameters for the oocyte donors and autologous oocyte patients. Ampules of gonadotropins used, number of oocytes, number of matured oocytes, number of fertilized embryos, endometrial thickness on the day of HCG and follicles ≥ 14 mm on the day of hCG were similar for both groups. However, basal antral follicle number and peak estradiol levels varied significantly between the two groups. There was a significant difference in the male partner age between oocyte recipients and IVF patients younger than 30 years using autologous oocytes, with males in the former group older by 7.8 years (33.7 ±7.6 vs. 41.5 ± 6.8) (p <0.0001).

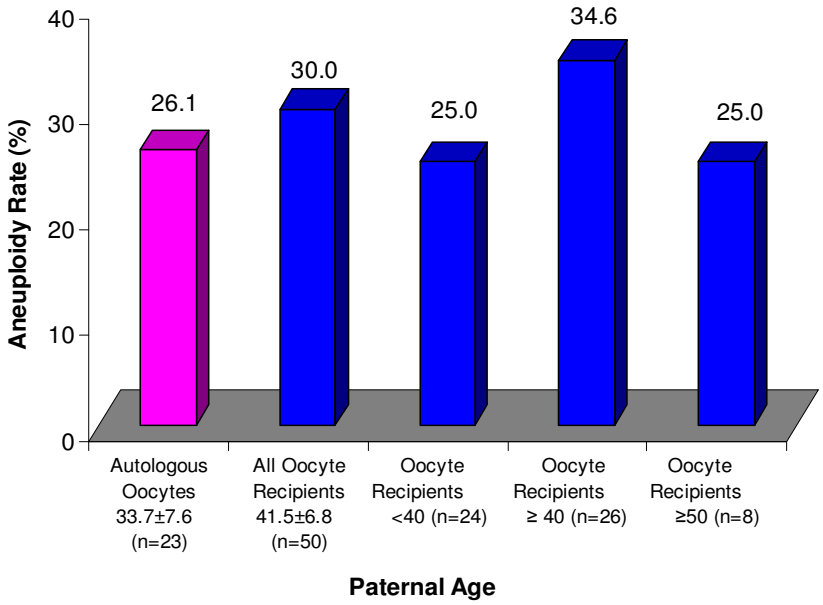

Figure 1. Aneuploidy rates following first trimester pregnancy loss in patients who conceived after oocyte donation and young patients who conceived with autologous oocytes plotted by paternal age.

However, no difference in aneuploidy rate was observed between these groups (30.0 vs. 26.1%) (p = 0.66; RR = 0.93; 95% CI (0.68 - 1.27)).

DISCUSSION

Since several studies have linked paternal aging to risk for miscarriage and adverse reproductive success rates, the mechanism for this has not been clearly delineated. Moreover, the study's hypothesis was that paternal aging would increase the aneuploidy rate for couples. This study design was unique in that oocyte donation was used as a model to control maternal age, thereby allowing the focus to be on the reproductive potential of the aging male.

Karyotypes of abortuses from a group of young female patients who miscarried following IVF with autologous oocytes were also examined. In both comparison groups, the mean age of the oocytes was less than 30 years. While controlling the effect of the female age, the study's data support that paternal age has little effect on aneuploidy rates.

Since it is possible that aneuploidy rates vary mostly at the extremes of male age, one limitation of this study is the small number of males older than 50. Secondary to the young age of the oocyte, it is uncommon for oocyte donor cycle participants to have genetic testing performed after a pregnancy loss. The same is true for autologous oocyte IVF cycles in young women under the age of 30. These factors contributed to the major limitation of this study (that is, its small sample size and power). Other limitations include the retrospective design of the study. Although no difference in the semen characteristics between the groups was found, this study did not control for the effect that male factor infertility may have on miscarriage or aneuploidy rates. When patients that underwent ICSI were compared to conventional IVF, no statistically significant difference in aneuploidy rates was seen.

Endometrial receptivity is an important recipient, which is a related determinant of the reproductive success for patients using donor oocytes. Therefore, it is important to note that the mean endometrial thickness was favorable in all groups (≥8 mm) and not significantly different in patients using autologous versus donor oocytes.

In summary, oocyte donation is a useful model in studying the influence of male aging on the reproductive potential of the couple. Based on these preliminary data, advancing male age seems to have a little impact on aneuploidy rates. However, further studies with a larger sample size are needed to elicit the mechanism by which male aging may contribute to adverse pregnancy outcome.

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