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ORIGINAL ARTICLE *Andrology*

Paternal age and assisted reproductive outcomes in ICSI donor oocytes: is there an effect of older fathers?

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STUDY QUESTION: Does paternal age affect semen quality and reproductive outcomes in oocyte donor cycles with ICSI?

SUMMARY ANSWER: Paternal age is associated with a decrease in sperm quality, however it does not affect either pregnancy or live birth rates in reproductive treatments when the oocytes come from donors <36 years old and ICSI is used.

WHAT IS KNOWN ALREADY: The weight of evidence suggest that paternal age is associated with decreasing sperm quality, but uncertainty remains as to whether reproductive outcomes are affected. Although developed to treat severe sperm factor infertility, ICSI is gaining popularity and is often used even in the presence of mild male factor infertility.

STUDY DESIGN, SIZE, DURATION: A retrospective cohort study spanning the period between February 2007 and June 2010. A total of 4887 oocyte donation cycles were included.

PARTICIPANTS/MATERIALS, SETTING, METHODS: Fertilization was carried out by ICSI in all cycles included, and the semen sample used was from the male partner in all cases. The association of male age with semen parameters (volume, concentration, percentage of motile spermatozoa) was analyzed by multiple analysis of covariance. The association of male age with reproductive outcomes (biochemical pregnancy, miscarriage, ongoing pregnancy and live birth rate) was modeled by logistic regression, where the following covariates were introduced: donor age, recipient age, semen state (fresh versus frozen) and number of transferred embryos (3 and 2 versus 1).

MAIN RESULTS AND THE ROLE OF CHANCE: We identified a significant relationship between paternal age and all sperm parameters analyzed: for every 5 years of age, sperm volume decreases by 0.22 ml ($P < 0.001$), concentration increases by 3.1 million sperm/ml ($P = 0.003$) and percentage motile spermatozoa decreases by 1.2% ($P < 0.001$). No differences were found in reproductive outcomes (biochemical pregnancy, miscarriage, clinical pregnancy, ongoing pregnancy and live birth) among different male age groups.

LIMITATIONS, REASONS FOR CAUTION: The use of donor oocytes, while extremely useful in highlighting the role of male age in reproductive outcomes, limits the generalization of our results to a population of young women with older male partners. No data were available on perinatal and obstetrical outcomes of these pregnancies. Most (75%) cycles used frozen/thawed sperm samples which might have introduced a bias owing to loss of viability after thawing. ICSI was performed in all cycles to control for fertilization method; this technique could mask the natural fertilization rate of poorer sperm samples. Furthermore, we did not use stringent ICSI indications; and our data are therefore not generalizable to cases where only severe male factor is considered. However, male patients were of different racial background, thus allowing generalizing our results to a wider patient base.

WIDER IMPLICATIONS OF THE FINDINGS: Our study suggests that paternal age does not affect reproductive outcomes when the oocyte donor is <36 years of age, indicating that ICSI and oocyte quality can jointly overcome the lower reproductive potential of older semen.

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Key words: male age / sperm / donor oocyte / ICSI / pregnancy rate

Introduction

In the developed world, the age of first-time parenthood has increased steadily during the last decades (Schmidt *et al.*, 2012). The effect of increased maternal age on reproduction has been widely studied, as it influences negatively pregnancy rate and oocyte quality while increasing the likelihood of aneuploidies in developing embryos (Hendershot, 1984; Munne *et al.*, 1995; Benadiva *et al.*, 1996). However, the effect of paternal age on both sperm characteristics and reproductive outcomes has been comparatively less studied in the general population, and there is no common consensus on its role in reproductive success. Moreover, it is still unclear what role the age of male gametes plays in assisted reproduction technologies (ARTs).

Multiple reports show a decline in seminal volume, motility and morphology with increasing paternal age (Dondero *et al.*, 1985; Centola and Eberly, 1999; Kidd *et al.*, 2001; Eskenazi *et al.*, 2003; Hellstrom *et al.*, 2006; Stone *et al.*, 2013). The inverse relation between sperm volume and advanced age appears clear (Dain *et al.*, 2011), with a calculated decrease of 0.03 ml per year in the total sperm volume in healthy men (Eskenazi *et al.*, 2003). Discrepancies, however, remain when considering sperm concentration; while some studies describe a decline with age (Auger *et al.*, 1995; Centola and Eberly, 1999; Aboulghar *et al.*, 2007; Luna *et al.*, 2009), others reported no association (Carlsen *et al.*, 1992; Whitcomb *et al.*, 2011) or even an increase in concentration (Irvine *et al.*, 1996; Andolz *et al.*, 1999).

Besides semen quality, and outside of the population of spontaneously conceiving individuals, it is important to evaluate the effect of paternal age in ART, as new knowledge may modify our clinical approach towards a couples' treatment.

Paternal age was found to have a detrimental effect on pregnancy rates in cycles of conventional IVF adjusted for maternal age (Klonoff-Cohen and Natarajan, 2004), as well as in conventional IVF cycles where the man was older than 40 and the woman was 35–37 years old (de La Rochebrochard *et al.*, 2006).

Other studies only considered ICSI when investigating the effect of male age. A study analyzing 821 cycles reported no influence of male age on pregnancy outcomes when women were younger than 36 years of age (Spandorfer *et al.*, 1998). However, another report with >1000 cycles, adjusted for maternal age, found a significant decrease in implantation rates with increasing paternal age in oligozoospermic patients and this same effect was not observed in normospermic patients (Ferreira *et al.*, 2010). Although ICSI have been developed mainly to treat severe male infertility, it is now acquiring a more widespread use because of its ability to increase the fertilization rate of the oocyte cohort; so far, a negative long-term effect of elective ICSI on the offspring has not been demonstrated; however, ICSI clearly limits the natural selection that the sperm undergoes during classical IVF.

As older men tend to have older partners, it is difficult to control for the effect of maternal age on oocyte quality. Oocyte donation cycles can overcome this limitation and have been used in a few studies (Gallardo *et al.*, 1996; Paulson *et al.*, 2001; Frattarelli *et al.*, 2008; Luna *et al.*, 2009; Whitcomb *et al.*, 2011). Unfortunately, the findings are still inconclusive, with some reports describing a significant decrease in both blastocyst and live birth rates in men over 50 years (Frattarelli *et al.*, 2008), or a decline in implantation rates in men over 60 years (Luna *et al.*, 2009), while others indicate a lack of effect of paternal age altogether (Gallardo *et al.*, 1996; Luna *et al.*, 2009; Whitcomb *et al.*, 2011).

With >5000 cycles analyzed, the current study is the largest reported so far; the main objective of our research is to assess whether paternal age affects pregnancy rates in oocyte donation cycles.

Materials and Methods

Study population

This study is a retrospective analysis of anonymized data from 4887 oocyte donation cycles which reached embryo transfer, collected at a single large fertility center in Spain from February 2007 to June 2010. This study did not require the approval of an Ethics Committee because the data were anonymous. Nevertheless, permission to conduct the study was sought and obtained from the Institutional Review Board.

Semen

The inclusion criterion for this study was oocyte donation cycles with ICSI as a fertilization method. Exclusion criteria were semen coming from a sperm donor, semen obtained by testicular biopsy and semen frozen prior to treatment for a medical condition. All patients received a doctor request for 3–5 days of abstinence before providing the semen sample used for evaluation and fertilization. The sample used for ICSI was either frozen (75%) or fresh (25%). All semen was initially frozen in a straw containing 1:1 volume of sample and Sperm CryoProtect II (Nidacon, Sweden). Frozen semen was deemed acceptable for ICSI if, at thawing, at least 35 000 progressive motile sperm could be found in a straw, corresponding to an initial 250 μl of ejaculate. If not acceptable, a fresh semen sample was used for ICSI. Regardless, all sperm samples were analyzed (volume, concentration, motility) using the standard laboratory procedures of the World Health Organization (1999, 2010).

Oocyte donors

All oocyte donors (age 18–35 years) had normally appearing ovaries at transvaginal ultrasound, an antral follicle count >8, and displayed a correct response to ovarian stimulation, i.e. a progressive and gradual increase in follicular sizes, concordant with FSH administration.

All donors were stimulated with exogenous gonadotrophins, while the pituitary suppression was based on two possible protocols: GnRH antagonists fixed from the sixth day of ovarian stimulation or GnRH agonists starting in the second phase of the preceding menstrual cycle. Regardless of the stimulation protocol, ovulation was triggered when three or more follicles ≥18 mm diameter were present on the ovaries. Ovulation trigger was performed with either 0.2 mg of the GnRH agonist Triptorelin (Decapeptyl® Ipsen, Pharma Biotech, France) or 250 μg hCG (Ovitrelle®, Merck, Germany) depending on the stimulation protocol.

Oocyte collection was performed 36 h after triggering by means of ultrasound-guided transvaginal follicular aspiration.

Recipients

The indications for oocyte donation were failed IVF cycles with own oocytes, low ovarian reserve, poor oocyte quality, genetic or chromosomal abnormalities transmissible to offspring and spontaneous or iatrogenic menopause. Women with menstrual cycles received an i.m. depot dose of GnRH agonist to suppress the pituitary. The estrogen endometrial preparation was started in the following cycle. An increasing dose of estrogens was used either orally or transdermally (from 2 to 6 mg, or from 75 to 150 μg, respectively) for a period of 15–40 days (average: 20.4; SD: 8; median: 17). This length of time was in all cases <49 days, the limit above which a significant decrease in uterine receptivity has been demonstrated (Soares *et al.*, 2005).

On the same day as oocyte retrieval in the donor, the recipient started with progesterone vaginally 400 mg/12 h as luteal phase support (Utrogestan® SEID, Spain or Progeffik®, Effik, Spain).

Embryo transfer was performed at Days 2, 3 or, much less frequently, 5 of development. On the same day as the transfer, the biologist scored the embryos following morphological criteria taking into account the number and symmetry of blastomeres, and the percentage of fragmentation (Ziebe *et al.*, 1997; Kamran *et al.*, 2012; Machtinger and Racowsky, 2013). In detail, the scoring of embryos on Days 2–3 was based on morphological criteria: number of cells, cell symmetry, pronuclei number per cell, embryonic fragmentation, presence/absence of vacuoles, presence/absence of cytoplasmic ring and embryo shape. The scoring system assigns a default score of 10 to each embryo, and then deducts points depending on the above-mentioned factors.

The treatments with estrogen and progesterone continued until the first assay of β-HCG in blood 15 days after transfer. In the case of a positive pregnancy test, the treatment was prolonged until Week 12 of pregnancy.

Statistical analysis

We analyzed the associations between male age and sperm parameters, embryo quality and reproductive outcomes. Semen parameters (volume, concentration, percentage of motile and immotile spermatozoa) and laboratory outcomes (including embryo quality) were analyzed by multiple analysis

Table I Cycle characteristics by brackets of male age.

Men	Total	<25	25–29	30–34	35–39	40–44	45–49	50–54	55–59	>60
Age										
N	5089	5	135	621	1443	1501	852	354	109	69
Mean	41.09	22.80	27.89	32.43	37.12	41.96	46.60	51.62	56.58	63.65
SD	6.77	1.10	1.21	1.34	1.41	1.40	1.40	1.37	1.34	4.56
Volume										
N	4680	3	122	564	1328	1383	782	327	104	67
Mean	3.67	2.17	3.88	3.94	3.92	3.75	3.39	2.95	2.89	2.46
SD	1.91	1.16	1.74	1.88	1.94	1.95	1.77	1.80	1.67	1.54
Concentration										
N	5067	5	135	620	1437	1497	846	351	107	69
Mean	92.14	66.11	90.86	86.78	87.53	87.62	97.62	127.60	99.05	80.64
SD	156.38	69.06	80.25	84.80	86.81	93.72	108.61	483.20	133.59	80.73
Motility A + B										
N	4988	5	132	607	1408	1472	844	347	107	66
Mean	19.39	24.20	25.27	20.05	19.82	19.68	18.44	17.17	16.50	14.35
SD	17.66	9.28	22.17	17.64	17.46	17.87	16.98	17.14	17.39	15.64
Semen state (fresh)										
N	5089	5	135	621	1443	1501	852	354	109	69
Percent	25.0	60.0	23.0	25.0	22.7	25.9	23.8	27.7	33.9	42.0
Fertilization rate										
N	5079	5	135	620	1441	1496	850	343	108	69
Mean	0.72	0.64	0.71	0.71	0.72	0.72	0.73	0.73	0.74	0.74
SD	0.22	0.26	0.25	0.23	0.22	0.22	0.22	0.22	0.20	0.24
Embryo transfer day										
N	5089	5	135	621	1443	1501	852	354	109	69
Mean	2.58	2.40	2.61	2.54	2.57	2.57	2.61	2.64	2.60	2.51
SD	0.64	0.55	0.74	0.64	0.62	0.63	0.64	0.73	0.76	0.58
Number of embryos transferred										
N	5089	5	135	621	1443	1501	852	354	109	69
Mean	1.91	1.80	1.92	1.93	1.92	1.91	1.91	1.91	1.91	1.83
SD	0.31	0.45	0.32	0.28	0.32	0.31	0.30	0.32	0.29	0.38
Embryo quality (blastocysts excluded)										
N	4951	5	128	607	1410	1464	829	337	103	68
Mean	8.57	8.57	8.57	8.57	8.57	8.57	8.57	8.57	8.57	8.57
SD	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05	1.05

of covariance (MANCOVA) adjusted for semen origin (fresh versus frozen) and donor age, and the mean adjusted effect was calculated.

Reproductive outcomes (biochemical pregnancy, miscarriage (before 14 weeks of pregnancy), ongoing pregnancy and live birth rate) were analyzed by logistic regression, where the following covariates were introduced: donor age, recipient age, semen origin (fresh versus frozen) and number of transferred embryos (1, 2 or 3). Male age was analyzed both as a continuous variable [odds ratios (OR) calculated for 5-year intervals] and as categorical variable. The adjusted ORs were obtained from the logistic regression. The statistical package R version 2.13.1 (The R Foundation for Statistical Computing ISBN 3-900051-07-0) was used for all analysis.

Results

Patient characteristics

Males included in the study were between 22 and 81 years old. The mean male age was 41.1 years (SD 6.7); the groups at the extremes included patients <30 years ($n = 131$) and ≥ 60 years old ($n = 65$). Details of the male characteristics across age brackets are presented in Table I, and the distribution of samples among ages is presented in Fig. 1.

All donors were healthy women between 18 and 35 years of age; they were on average 26.4 years old (SD 4.3), and had an average BMI of 22.9 kg/m^2 (SD 3.2).

The mean age of the recipients was 40.7 years (SD 4.7, range: 22–50). The relative distribution of donor and recipient characteristics for brackets of male age are presented in Table II.

Effect of paternal age on sperm parameters and laboratory outcomes

Data on semen were available for 4353 men. Paternal age affected negatively all semen and laboratory parameters, with the exception of semen concentration (Fig. 2). Semen volume decreased significantly with male age, with a mean reduction of 0.22 ml every 5 years [95% confidence interval (CI) 0.17–0.26] ($P < 0.001$). Semen concentration increased significantly with a mean increase of 3.1 million sperm per ml every 5 years of age [95% CI 1.1–5.2] ($P = 0.003$). The percentage of motile spermatozoa significantly decreased, with a mean reduction of 1.2% every 5 years of age [95% CI 0.84–1.15] ($P < 0.001$) (Fig. 3). An additional analysis based on total motile sperm count was carried out, with results similar to those obtained with percentage motile sperm (data not shown).

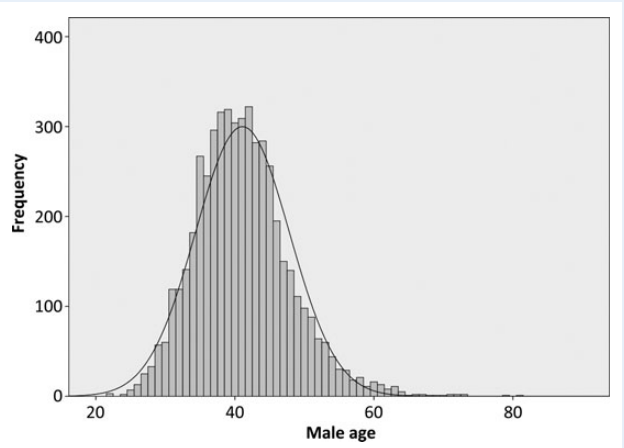

Figure 1 Distribution of age of men in this study.

We then evaluated the effect of male age on the average morphological score of the embryos transferred to the recipient, which we took as an indication of the quality of the cohort of embryos generated. There was no significant association between male age and the average morphological score, with a mean decrease of 0.02 [95% CI 0.002 to -0.044] ($P = 0.07$).

Effect of paternal age on reproductive outcomes

Two embryos were transferred in 89.6% of the cycles analyzed. Single embryo transfer was performed in 9.5% of the cycles, while 3 embryos were transferred in 44 cycles (0.9%). Transfer of three embryos was performed only after multiple failed attempts with previous donation cycles, and in recipients younger than 45 years old.

Embryos were usually transferred on Day 2 (48.1%) or Day 3 (49%) of *in vitro* development; in 11 (0.2%), 129 (2.6) and 1 (0.02%) cycle embryos were transferred at Days 4, 5 or 6, respectively.

A biochemical pregnancy was observed in 2336 cases (47.8%) and ongoing pregnancy was observed in 1928 (39.5%) cases. A miscarriage occurred in 408 cases (8.3%). A live birth was achieved in 1899 (38.8%) of the transfers. A total of 643 transfers (13.2%) resulted in more than one gestational sac, with 640 cases of 2 sacs and 3 cases of 3 sacs. In 586 (12%) of the transfers, more than one embryo with positive fetal heartbeat was detected (575 twins, 10 triplets and 1 quadruplet). After embryo reduction or spontaneous miscarriage, at second trimester ultrasound (10.0–13.6 weeks) there were 497 (10.2%) ongoing twin pregnancies, and 470 of them gave live births (9.6%). The rest of live births were singletons.

Male age was not associated with any of the reproductive outcomes analyzed (OR for a 5-year interval): biochemical pregnancy rate [1.0; 95% CI 0.96–1.05] ($P = 0.91$); miscarriage [1.06; 95% CI 0.94–1.03] ($P = 0.52$); ongoing pregnancy rate [0.98; 95% CI 0.94–1.033] ($P = 0.52$) and live birth rate [0.98; 95% CI 0.94–1.03] ($P = 0.52$) (Fig. 4 and Supplementary data, Table SI). Male age remained non-significantly associated with the reproductive outcomes when male age was analyzed as a categorical variable (Supplementary data, Table SII).

Moreover, the distribution of male age was normal in the 264 cycles with a fertilization rate $\leq 20\%$, indicating no effect of male age on the cycles not reaching transfer (Supplementary data, Fig. S1).

The results show that morphological embryo score and reproductive outcomes were only associated with the number of embryo transferred, and not with paternal age or any other variable analyzed. We did not find an effect of the state of sperm used (fresh or frozen) or recipient age on any of the reproductive parameters analyzed.

Discussion

This large retrospective study evaluated the influence of paternal age on semen parameters and reproductive outcomes in oocyte donor cycles with ICSI as fertilization technique. Both ICSI and donor oocyte cycles are increasingly resorted to in the ART field in order to increase the fertilization rate, which might convert into a higher cumulative pregnancy

Table II Recipient and oocyte donor characteristics by brackets of male age.

	Total	<25	25–29	30–34	35–39	40–44	45–49	50–54	55–59	>60
Recipient										
Age										
N	5089	5	135	621	1443	1501	852	354	109	69
Mean	40.74	31.00	35.01	37.04	39.54	41.55	42.84	44.21	44.35	44.14
SD	4.76	9.38	7.94	5.82	4.22	3.26	3.41	3.74	3.68	3.56
BMI										
N	4972	5	127	614	1411	1465	833	346	104	67
Mean	23.38	26.99	24.06	23.18	23.16	23.49	23.65	22.89	24.00	23.99
SD	4.05	3.74	3.95	4.28	3.96	4.02	4.17	3.64	4.35	3.82
Donor										
Age										
N	5047	5	134	614	1428	1492	844	352	109	69
Mean	26.40	25.00	26.51	26.16	26.46	26.37	26.33	26.52	26.91	27.04
SD	4.30	4.58	4.26	4.39	4.34	4.26	4.30	4.18	4.26	4.43
BMI										
N	5005	5	134	605	1417	1483	837	349	108	67
Mean	22.87	24.58	23.21	22.82	22.79	22.92	22.88	22.91	22.80	22.93
SD	3.22	3.20	3.09	3.16	3.23	3.23	3.22	3.37	3.24	3.10

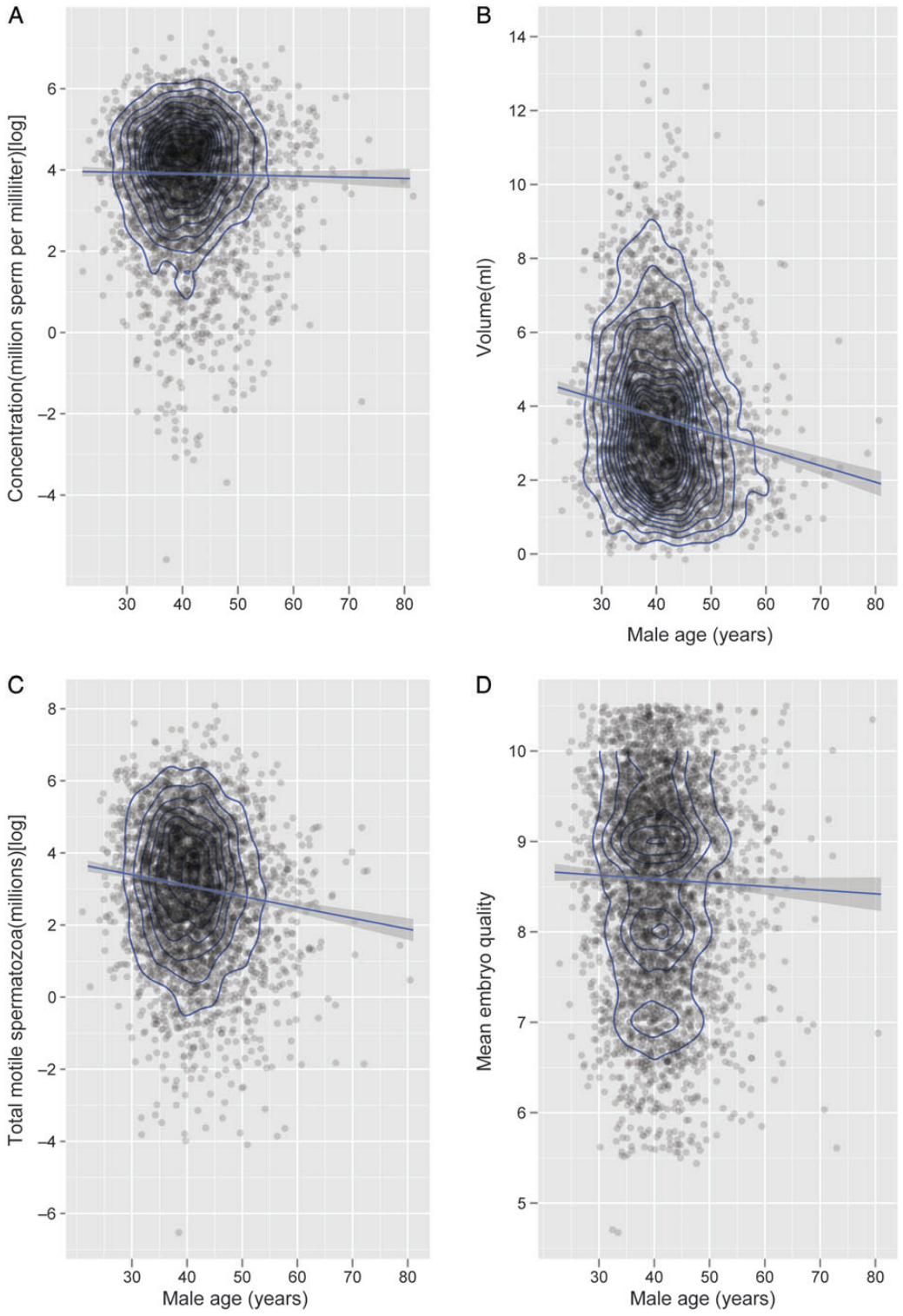

Figure 2 Distribution of human semen parameters and embryo quality across the sample population by male age; *x*-axis: male age. (**A**) Semen concentration (millions/ml), (**B**) semen volume (ml), (**C**) percentage of motile spermatozoa and (**D**) mean quality of the transferred embryos. The *y*-axis shows the average scores for embryos that were transferred in that cycle. The embryos chosen had the highest score in their cohort. The line in each panel represents the trend line for the variable distribution. The World Health Organization 2010 lower reference limits for semen characteristics are (mean (range): semen volume (ml): 1.5 (1.4–1.7); semen concentration (10^6/ml): 15 (12–16); total motility (%): 40 (38–42).

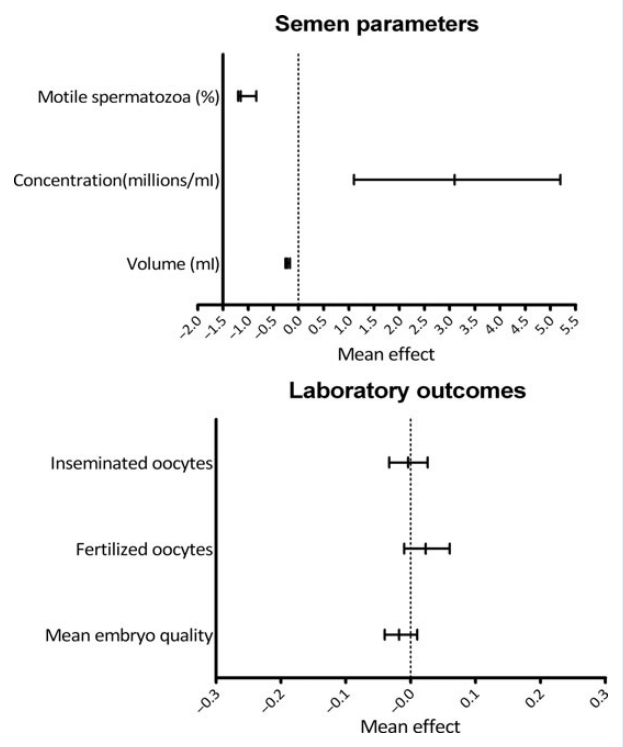

Figure 3 Adjusted effect (mean difference) of male age on semen parameters and laboratory outcomes.

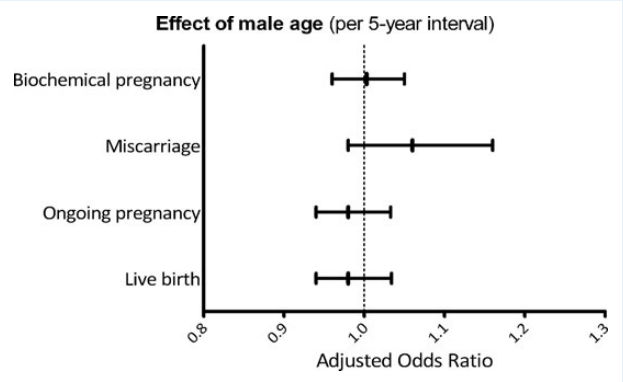

Figure 4 Adjusted effect (OR) of male age on reproductive outcomes.

rate per cycle, and as a response to the increasingly older female age in couples with a gestational desire. Although donor cycles in countries such as Spain, where female donor age is limited to 35 years by law, are highly successful, we should not forget the potential for more frequent obstetrical complications in older mothers. Our results show that male age is significantly associated with a lower seminal volume and percentage of motile spermatozoa. These findings are consistent with previous reports (Kidd *et al.*, 2001; Eskenazi *et al.*, 2003).

Interestingly, we found an increase in sperm concentration with male age. Given the large number of cases considered and the medical instruction to maintain 2–5 days of abstinence before providing the sample used for analysis, we do not believe that this result is due to chance. While abstinence interval might have been longer for older men, when asked 95% of men reported to have complied with the medical instructions on abstinence. Rather, we interpret it to be a function of a diminished seminal volume, which may or may not be accompanied by a lower spermatogenic output. Because of the characteristic of our patient base (referral patients often traveling from far away to reach the clinic), 75% of the cycles analyzed were carried out with frozen/thawed sperm samples. We recognize that the freezing and thawing might have introduced a bias, as there is a recognized loss of viability in semen after thawing; however, our statistical analysis did not report a significant effect of semen cryopreservation on reproductive outcomes.

The 25% of cycles with fresh sperm sample were distributed homogeneously among all age groups; regardless, reproductive outcomes have been shown to be comparable using ICSI with fresh or frozen sperm samples (Huang *et al.*, 2004; Kalsi *et al.*, 2011).

ICSI was used in all cases included in this study, therefore, it is possible that we minimize the detrimental effect on fertilization of the semen samples with the lowest quality; however, it has been repeatedly shown that once a cycle of stimulation has reached the embryo transfer stage, the fertilization technique does not change the cycle reproductive outcome (van der Westerlaken *et al.*, 2006; Shi *et al.*, 2010; Johnson *et al.*, 2013).

Regarding the fertilization rate and mean embryo quality, our results show no differences among male age groups. We evaluated the mean embryo quality based on a morphological assessment (Coroleu *et al.*, 2006); this evaluation method has limitations, as its correlation with implantation is not linear for all scores. However, morphological score has been, for a long time, the most widespread technique used to select the embryos for transfer (Ren *et al.*, 2012; Machtinger and Racowsky, 2013). We found a positive relation between the morphological score and the fertilization success rate. Comparing the day of transfer (Day 3 versus 2) our results show a significant correlation between transferring embryos on Day 3 of development and live birth rate. Our results are in disagreement with other reports (Laverge *et al.*, 2001), where both implantation and pregnancy rates were found to be comparable for Day 2 and 3 transfer; the discrepancy is possibly due to the study of cycles using the patient own oocytes.

In agreement with other reports analyzing a lower number of cases (Gallardo *et al.*, 1996; Paulson *et al.*, 2001; Bellver *et al.*, 2008; Whitcomb *et al.*, 2011), we found no correlation between male age and any of the reproductive outcomes. However, two studies found a significant decrease in blastocyst rate in oocyte donation cycles in males older than 50 years (Frattarelli *et al.*, 2008; Luna *et al.*, 2009). One possible explanation for this apparent discrepancy might be related to the strong selection effect that culturing the embryo to blastocyst stage has (~30% do not routinely reach blastocyst stage), or to a bias in the population to whom blastocyst culture is proposed. If this were true, the analysis of Day 2 and 3 transfers might reflect more closely the effect of semen age on the developing embryo.

The large sample size of the current study, together with the standardization of the donor stimulation protocols, the recipient endometrial preparation and the sperm sample evaluation provides enough scientific weight to conclude that paternal age does not play a significant role in determining the results of ICSI when the oocyte is young and healthy.

Regardless of the results from our group and others on reproductive outcomes in ART and male age, it is undeniable that natural conception and pregnancy rates after minor manipulations, such as intrauterine insemination, decrease with increasing male age, even after controlling for maternal age. We think that the discrepancy in our data could be due to two main reasons. On the one hand the oocytes in donor cycles are from young women, often of proven fertility, either because they have had their own children or through recipients in previous donation cycles. The health of the oocyte could improve the outcome of using a less than perfect sperm, as has been suggested in the case of DNA double-strand breaks. On the other hand, sperm preparation, and especially ICSI, allows for a strong selection of sperm and a forced entry into the oocytes, thus clearing some of the physiological hurdles which might become critical as sperm age.

We do recognize some weakness in our study, and specifically the lack of detailed perinatal outcomes and obstetrical information on the pregnancies. Although our goal was to study the effect of male age on reproductive outcomes, recent evidence suggests a role of the father's age in post-natal development and health of the offspring (Kong *et al.*, 2012; d'Onofrio *et al.*, 2014). Further studies will be necessary to identify possible post-natal and developmental alterations in children of older fathers.

Supplementary data

Supplementary data are available at http://humrep.oxfordjournals.org/.

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Authors' roles

R.B.: data collection, manuscript preparation and revision, D.G.: study design, data collection, data analysis and manuscript revision. A.O. and F.P. expert knowledge. R.V.: study design, study coordination, expert knowledge, manuscript writing and revision and V.V.: study design and expert knowledge.

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Conflict of interest

None declared.

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