Article

Influence of paternal age on assisted reproduction outcome

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Abstract

There has been an increasing tendency to delay parenthood in developed countries in recent years, and there is not enough information available regarding the effect of this on fertility. The aim of this work was to determine the role of paternal age on the outcome of assisted reproduction. A retrospective study was designed comprising a total of 2204 intrauterine insemination (IUI) cycles, 1286 IVF cycles and 1412 IVF cycles with donated oocytes during the period 2000 to 2006. Male mean age was 34.3 years (range 25–56) for IUI, 34.8 years (range 19–62) for IVF and 41.10 years (range 25–71) for ovum donation cycles. Statistics revealed no differences regarding pregnancy and miscarriage rates when the results were compared among age groups. In standard IVF and ovum donation cycles there was no clear association between embryo quality and paternal age. There was no significant relationship between male age and implantation rate. So far this is the largest study concerning the relevance of male age in assisted reproduction. As confirmed by the present data, the effect of the age of the male in the range studied is irrelevant. This finding contributes to the information that can be provided to infertile couples.

Keywords: *IUI, IVF/ICSI, male age, ovum donation, reproductive outcome, sperm quality*

Introduction

Advanced paternal age has been related to infertility, increased risk of miscarriage, obstetric problems and several defects in offspring. Increased risks of Caesarean section (Tang *et al*., 2006), low birth weight (Reichman *et al*., 2006), preterm birth (Zhu *et al*., 2005; Astolfi *et al*., 2006), pre-eclampsia (Harlap *et al*., 2002), congenital malformations (Zhu*et al*., 2005), Down syndrome (Fisch *et al*., 2003; Zhu *et al*., 2005), fetal and neonatal mortality (Gourbin, 2005), autism (Reichenberg *et al*., 2006), epilepsy (Vestergaard *et al*., 2005), breast cancer (Choi *et al*., 2005), type 1 diabetes (Cardwell *et al*., 2005), and schizophrenia (Tsuchiya *et al*., 2005) have been recently reported in pregnancy and offspring of older fathers.

Age 40 years has been proposed as the cut-off age after which some reproductive and offspring complications increase in men (Choi *et al*., 2005; Astolfi *et al*., 2006; de La Rochebrochard *et al*., 2006a,b; Reichenberg *et al*., 2006). However, a trend towards impaired outcomes seems to start at 20 to 30 years old (Tang *et al*., 2006). Some of these pathological conditions are also increased by advanced maternal age (Fisch *et al*., 2003; Astolfi *et al*., 2006 de La Rochebrochard E *et al*., 2006; Tang *et al*., 2006).

Genetic changes in the germ cells of older men, including de-novo mutations in sperm cells or alterations in genetic imprinting, are presumed to underlie most of the described complications in the offspring (Harlap *et al*., 2002; Choi *et al*., 2005; Tsuchiya *et al*., 2005; Zhu *et al*., 2005; Reichenberg *et al*., 2006). They can be induced by biological or environmental factors (Zhu *et al*., 2005). A higher frequency of sperm chromosome aberrations, as a result of increased non-

disjunction, acentric fragments and complex radial figures, has also been described in older men (Sartorelli *et al.*, 2001).

Regarding fertility, men ≥40 years old have shown a delay in pregnancy onset (failure to conceive within 12 months), more difficulties in having a baby (failure to conceive within 12 months or pregnancy not resulting in a live birth) (de la Rochebrochard *et al.*, 2006), and an increased risk of spontaneous abortion (Kleinhaus *et al.*, 2006), which seems to be more related to first trimester losses (Slama *et al.*, 2005). Some recent reports have highlighted a continuous decrease in sperm motion and semen volume between 22 and 80 years of age, with no evidence of a threshold (Sloter *et al.*, 2006), as well as lower total sperm counts and sperm morphology (Auger and Juannet, 2005).

Similarly, pregnancy chances after intrauterine insemination (IUI) (Mathieu *et al.*, 1995) and IVF or gamete intra-Fallopian transfer (GIFT) (Klonoff-Cohen and Natarajan, 2004) have shown to be lower with advanced male age. However, there is still controversy on this issue because relatively few children are born to older fathers and some reports do not find a poorer prognosis when assisted reproduction technologies are employed in older men (Spandorfer *et al.*, 1998).

The aim of the present work was to assess the influence of paternal age on the reproductive outcome of young women after assisted reproduction [IUI, IVF/intracytoplasmic sperm injection (ICSI) or ovum donation]. A subgroup of ovum donation was included, in which differences in female factor are significantly reduced.

Materials and methods

Institutional approval

This project was approved by the Institutional Review Board on the use of human subjects in research at the Instituto Valenciano de Infertilidad, and complies with the Spanish Law of Assisted Reproductive Technologies (Lacadena, 2006).

Patients

The databases of the clinic, Instituto Valenciano de Infertilidad in Valencia (Spain), were retrospectively searched for assisted reproduction procedures (IUI, standard IVF/ICSI cycles and ovum donation IVF/ICSI cycles) performed during the period from January 2000 to October 2006, yielding a total number of 4902 cycles from 3669 men, whose histories were studied.

IUI

A total number of 2204 IUI cycles (971 patients) were obtained from the data files. The inclusion criteria were: women under 38 years old, tubal patency demonstrated by hysterosalpingography, normal uterine scan, normal basal hormonal concentrations, body mass index (BMI) <27 kg/m^2, and absence of polycystic ovarian syndrome and endometriosis.

IUI was performed as previously described (Muriel *et al.*, 2006a). Couples were categorized depending on male age in percentiles of similar width (approximately 10% width). The following groups were created: up to 30, 31, 32, 33, 34, 35, 36, 37, 38–39 and more than 39 years. Obviously, because age is a truncated variable, all the groups were not exactly the same size, although they were quite similar.

IVF

In IVF, 1286 cycles (1286 patients) and 17,746 oocytes were considered. The inclusion criteria were: women under 38 years old, BMI <27 kg/m^2, normal uterine scan, normal basal hormonal concentrations, first IVF cycle and absence of polycystic ovarian syndrome, hydrosalpinx or endometriosis. The indications for IVF were IUI failure, tubal pathology or infertility of unknown origin. IVF was considered only in couples with absence of severe oligozoospermia (<5 × 10^6/ml). IVF procedures were carried out as previously described (Muriel *et al.*, 2006b). Implantation, pregnancy and miscarriage rates were assessed, as well as different parameters related to embryo quality (number of cells, symmetry and percentage of fragmentation in day 2 and day 3 embryos) (Muriel *et al.*, 2006a). Couples were also categorized depending on the male age in percentiles (approximately 10% width), creating the following groups: up to 30, 31–32, 33, 34, 35, 36, 37, 38, 39–40 and more than 41 years. Because age is a truncated variable, all the groups were not exactly of the same size, although they were quite similar.

Ovum donation

In oocyte donation, 1412 IVF cycles (1412 patients) and 14,621 oocytes were considered. The median age of the donor population was 25.9 years (SD = 4.1) and 40.7 years (SD = 3.19) for oocyte recipients. Indications for ovum donation were: low ovarian response, premature ovarian failure, advanced female age, menopause and poor oocyte quality. The inclusion criteria were: BMI <27 kg/m^2, normal uterine scan, first oocyte donation cycle and absence of endometriosis. Cycles were studied only in couples with absence of severe male factor (less than 5 × 10^6/ml). The removal of severe male factors avoided any bias when categorizing the patients depending on the male age. As a retrospective study, if some of the groups were unbalanced in the proportion of severe male factor, this would alter the clinical results, masking the real effect. Couples were also categorized depending on the male age in percentiles (approximately 10% width), creating the following groups: up to 34, 35–37, 38, 39–40, 41, 42, 43–44, 45–46, 47–49 and more than 49 years. Because age is a truncated variable all the groups were not exactly of the same size, although they were quite similar.

Semen analysis

The clinical features of all of the patients studied (IUI, IVF and oocyte donation) were analysed. Sperm samples were examined and prepared as previously described (Meseguer *et al.*, 2004, 2006).

Statistical analysis

Correlation between basic sperm parameters and paternal age values (in the whole age range) were performed by linear regression analysis.

Chi-squared tests followed by Bonferroni's correction (multiplying *P*-value by number of comparisons performed) were employed to compare pregnancy and miscarriage rates among groups, and then, the results were stratified depending on the women's age (less than 30, from 31 to 35, from 36 to 38 years). In IUI, cumulative pregnancy rates in four cycles were compared by Kaplan–Meier survival analysis, followed by log rank, Breslow, and Tarone–Ware tests to evaluate the equality of the survival distributions for the different levels of the factor.

Fertilization and implantation rates, triploid rate and embryo quality parameters (number of blastomeres, percentage of embryo fragmentation, total number of frozen embryos on days 2 and 3) were related to paternal age by linear regression analysis.

Finally, taking into consideration that the categorization of a continuous variable, like paternal age, reduces statistical power, a logistic regression analysis was also performed in which the effect of paternal age on pregnancy chances was quantified. A model was developed in which maternal age and maternal BMI were included as confounding factors. The significance of the model was calculated by the omnibus test (likelihood ratio) and the uncertainty explained by the model was evaluated by Negelkerke R^2. Odds ratio (OR) of the effect of 1 year older on pregnancy outcome was expressed, together with 95% confidence interval (CI) R^2 and significance. Receiver operating characteristic (ROC) curves were employed to test the predictive value of paternal age on pregnancy achievement. In these curves, the effects of maternal age and BMI were also taken into consideration as confounding factors that could be affecting the predictive properties of paternal age. ROC curve analysis provides AUC (area under the curve) values; this value lies between 0.5 and 1.0 and can be used as a measurement of the global exactitude of the model. 1.0 is considered as a perfect diagnostic value and 0.5 is considered an absence of diagnosis power. An AUC over 0.8 is considered acceptable from a predictive point view. Significance was assumed at $P < 0.05$.

Statistical analysis was performed using the Statistics Package for Social Sciences (SPSS Inc., Chicago, IL, USA) and MedCalc Software (Ghent, Belgium).

Results

In the IUI population male mean age was 34.3 years (95% CI 34.2–34.5), ranging from 25 to 56 years. Mean motile sperm count in the ejaculated sample was 94.43 × 10^6/ml (95% CI 90.81–98.06), and 12.29 × 10^6/ml (95% CI 11.80–12.78) in the post-swim-up sample. A mean number of 2.3 cycles (95% CI 2.21–2.36) per couple were performed. BMI was 22.43 (95% CI 21.65–23.21). Global pregnancy rate per cycle was 18.0% (*n* = 396) (95% CI 16.4–19%) and cumulative pregnancy rate in four consecutive cycles was 18.89 (first), 33.44 (second), 44.44 (third) and 52.79% (fourth). Miscarriage rate was 21.5% (*n* = 85) (95% CI 17.3–25.6), while multiple pregnancies accounted for a 21.7% (*n* = 86) of the pregnancies (95% CI 17.6–25.6).

In the IVF/ICSI population, male mean age was 34.8 years (95% CI 34.6–35.03), ranging from 19 to 62 years. Mean motile sperm count in the ejaculated sample was 72.75 × 10^6ml (95% CI 68.75–76.74), and 3.94 × 10^6ml (95% CI 3.17–4.70) in the post-swim-up sample. BMI was 21.94 (95% CI 21.67–22.21). Number of transferred embryos was 2.26 (SD = 0.84). Pregnancy rate per cycle was 52.3% (95% CI 49.5–55.1) (*n* = 672). Miscarriage rate was 15.3% (95% CI 12.60–18.10) (*n* = 103), while multiple pregnancies accounted for 35.7% (*n* = 240) of total pregnancies (95% CI 32.1–39.5).

In the ovum donation population, male mean age was 41.40 years (95% CI 41.08–41.70), ranging from 25 to 71 years. Mean motile sperm count in the ejaculated sample was 19.51 × 10^6ml (95% CI 17.63–21.39), and 1.08 × 10^6ml (95% CI 0.64–1.52) in the post-swim-up sample. BMI was 22.46 (95% CI 22.11–22.81). Number of transferred embryos was 2.19 (SD = 0.59). Pregnancy rate was 51.5% (95% CI 48.9–54.1%) (*n* = 727). Miscarriage rate was 18.6% (95% CI 16.0–21.3) (*n* = 135), while multiple pregnancies accounted for 35.9% of pregnancies (95% CI 33.4–38.1) (*n* = 261).

Semen parameters and paternal age

Linear regression analysis was performed to study the effect of the quantitative variable, paternal age (years), on quantitative sperm parameters obtained from basic sperm analysis in order to identify any association between them. The study was also applied to the whole range of age of the population: 19–71 years. A significant negative association was found between male age and volume, concentration, motility and total progressive motility in fresh spermatozoa and after swim-up (**Table 1**). From all the Pearson's coefficient values presented, only two could be considered clinically relevant because they were around or over 0.3. Those parameters were fresh ejaculate progressive motility and ejaculate volume.

Additionally, another analysis was performed with the database, looking at the distribution of severe male factor

Table 1. Linear regression analysis (*r* value) of male age and basic semen parameters before and after swim-up.

Semen parameter	*Ejaculate*	*Post swim-up*
Volume	–0.262	–0.147
Concentration	–0.079	–0.204
Progressive motility	–0.324	–0.224
Total progressives	–0.218	–0.161

All values show a significant linear relationship, $P < 0.05$.

depending on male age. A total of 2193 cases of severe male factor were detected in that period of time. Firstly, binary logistic regression analysis demonstrated no effect of male age on the diagnosis of severe male factor; exp(B) = 1.007, 95% CI (0.999–1.0015). The average age and CI of patients with severe male factor who underwent IUI were also analysed (35.7 years, 95% CI 34.05–37.84), ICSI (35.3 years, 95% CI 35.1–35.9) and oocyte donation (40.7 years, 95% CI 40.16–41.33). The values are similar to those for the groups analysed, and the 95% CI included those mean values of the study population for the IUI, IVF/ICSI and oocyte donation groups. From these results, it is concluded that avoiding severe male factor did not bias the results of this paper, because severe male factor was associated with all ages.

Paternal age and IUI results

Chi-squared analysis and linear-by-linear association analysis (in order to analyse linear tendencies) revealed no differences regarding pregnancy and miscarriage rates when the results were compared among age groups (**Figure 1**).

Stratified data analysis also confirmed these findings, and no significant difference in pregnancy rates were observed according to maternal age (**Table 2**).

Moreover, cumulative pregnancy rates in four consecutive IUI cycles were not significantly different. Despite the large differences observed between some of the groups (i.e. from 38 to 63%), the absence of association between these differences and paternal age was shown as no significant differences were found in survival curve analysis (**Figure 2**).

The predictive value of paternal age (incorporating the effect of maternal age and their BMI) were estimated by a ROC curve analysis (**Figure 3**), AUC = 0.600 (95% CI 0.533–0.668).

A logistic regression analysis was performed in order to quantify the effect of male age on the IUI outcome (**Table 3**). A significant association was observed, but this is probably not clinically important: this is based on R^2 (Negerlkerke) = 0.036; this means that only 3.6% of the variations in the pregnancy rate in this group of patients are reproduced or explained by this model, while the rest of the variations are uncertain.

Finally, only eight men who underwent IUI were more than 50 years old, and for three men the treatment resulted in a pregnancy (37.5%).

Paternal age and standard IVF/ICSI results

No association was found between fertilization rate and male age. In addition, there were no important correlations between embryo division and fragmentation 48 and 72 h after fertilization and paternal age. This is based on the *r* values; in statistics focused on biological correlations *r* values over 0.3 are considered acceptable, while in the present study correlations of $r = 0.036$ and $r = 0.010$ respectively were found. These are extremely low and provide only a weak explanation for the variations in embryo quality (**Table 4**).

Chi-squared analysis and linear-by-linear association analysis (in order to analyse linear tendencies) revealed no differences regarding pregnancy and miscarriage rates when the results were compared among age groups (**Figure 4**). Interestingly, of the six men who underwent these treatments who were more than 50 years old, only one pregnancy was achieved (16.67%).

There was no correlation between male age and implantation rate (**Table 4**). Pregnancy rate was similar when male age was considered according to female age (<30; 31–35; 36–38) (**Table 5**).

The predictive value of paternal age (incorporating the effect of maternal age and their BMI) were estimated by a ROC curve analysis (**Figure 3**), AUC = 0.541 (95% CI 0.479–0.603).

A logistic regression analysis was performed in order to quantify the effect of male age on IVF/ICSI outcome (**Table 3**). No significant association was observed.

In addition, there were no differences in multiple pregnancies among male age groups (data not shown).

Paternal age and oocyte donation cycles

The effect of paternal age on fertilization was analysed, and no differences were observed. As presented in **Table 4**, a positive correlation was observed between embryo fragmentation 48 and 72 h after fertilization and paternal age, although *r* values were not clinically relevant. The correlations were below 0.3, being $r = 0.028$ and $r = 0.027$ respectively. These values are extremely low and provide only a weak explanation for the variations in embryo quality (**Table 4**). No associations with other embryo parameters were detected (**Table 4**).

Chi-squared analysis and linear-by-linear association analysis (in order to analyse linear tendencies) revealed no differences regarding pregnancy and miscarriage rates when the results were compared among age groups (**Figure 5**). A considerably larger number of men over 50 years old ($n = 125$) underwent these treatments (compared with IUI or IVF/ICSI); of these 61 achieved a pregnancy (48.8%). Implantation rate was not correlated with paternal age.

The predictive value of paternal age were estimated by a ROC curve analysis (**Figure 3**), AUC = 0.563 (95% CI 0.46–0.657).

Finally, a logistic regression analysis was performed in order to quantify the effect of male age on ovum donation outcome (**Table 3**). A significant association were observed, but probably this was not clinically important. This is based in R^2 (Negerlkerke) = 0.04; this means that only 4% of the variations in the pregnancy rate in this group of patients were reproduced or explained by this model, while the rest of variations were uncertain.

Discussion

Older male age has been related to infertility and poorer outcome in assisted conception. One of the mechanisms

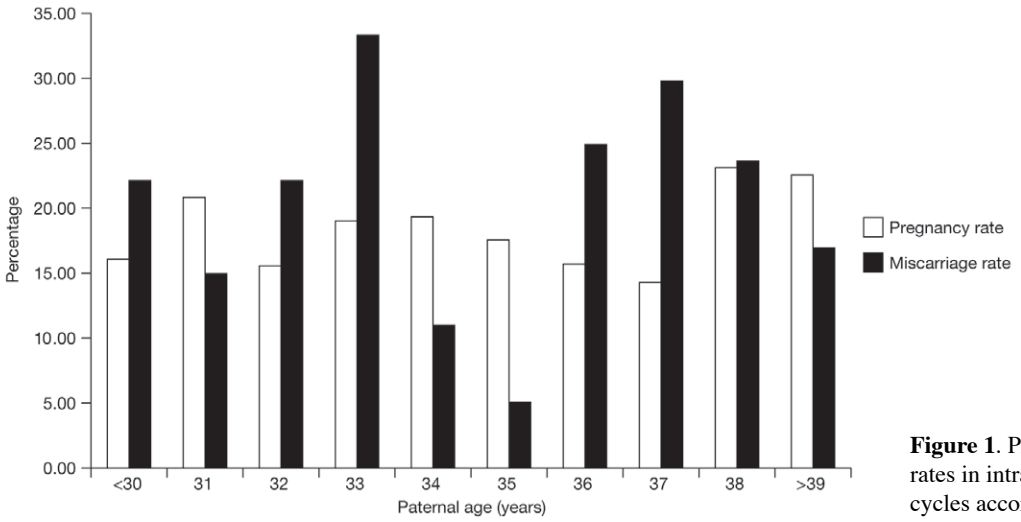

Figure 1. Pregnancy and miscarriage rates in intrauterine insemination (IUI) cycles according to male age.

Table 2. Pregnancy rates in intrauterine insemination (IUI) according to male and female age.

Female age (years)	*Male age (years)*									
	<30	*31*	*32*	*33*	*34*	*35*	*36*	*37*	*38*	*>39*
< 30	15.4 (22/143)	22.1 (19/86)	20.5 (18/88)	24.8 (29/117)	28.4 (27/95)	18.2 (18/99)	22.0 (18/82)	13.6 (8/59)	30.1 (25/83)	32.5 (25/77)
31–35	20.4 (20/98)	36.4 (16/44)	12.3 (7/57)	29.5 (23/78)	23.5 (16/68)	26.0 (19/73)	5.6 (3/54)	20.8 (11/53)	34.5 (20/58)	15.9 (7/44)
36–38	19.7 (15/76)	0.0 (0/29)	16.0 (8/50)	23.0 (14/61)	14.7 (10/68)	26.5 (13/49)	17.5 (10/57)	20.0 (11/55)	28.0 (14/50)	43.5 (20/46)

Values in parentheses are number of pregnancies/number of cycles.

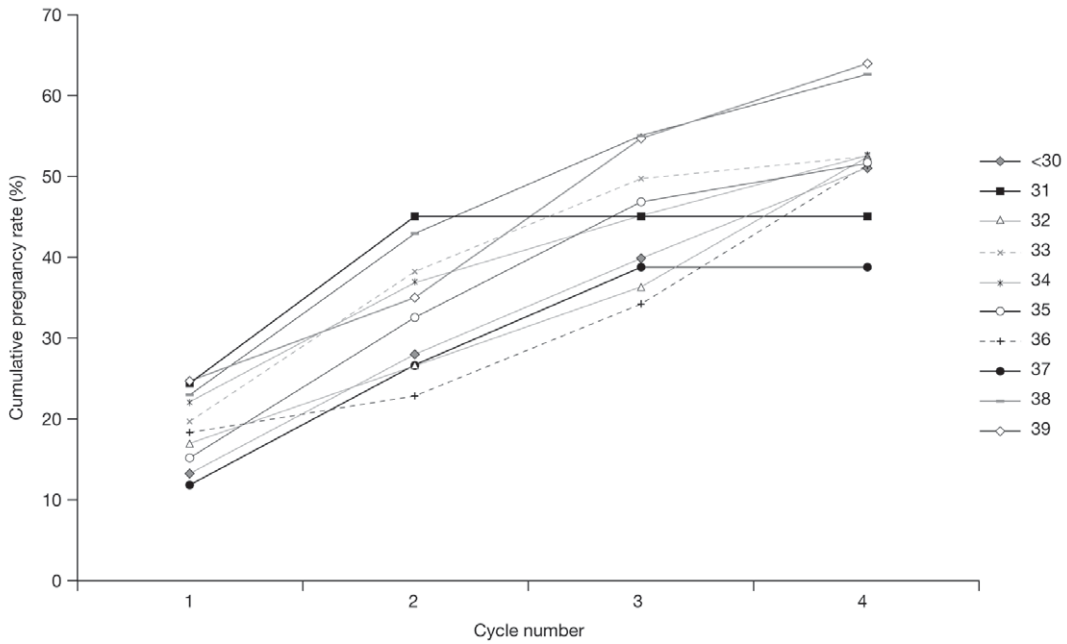

Figure 2. Cumulative pregnancy rates in relation to different age groups in intrauterine insemination procedures. Cumulative pregnancy rates in four cycles were compared by Kaplan–Meier survival analysis, followed by log rank, Breslow, and Tarone–Ware tests to evaluate the equality of the survival distributions for the different levels of the factor. Similar cumulative pregnancy rates curves were observed for different age groups (*log* rank 0.3252, Breslow 0.5586, Tarone–Ware 0.4574).

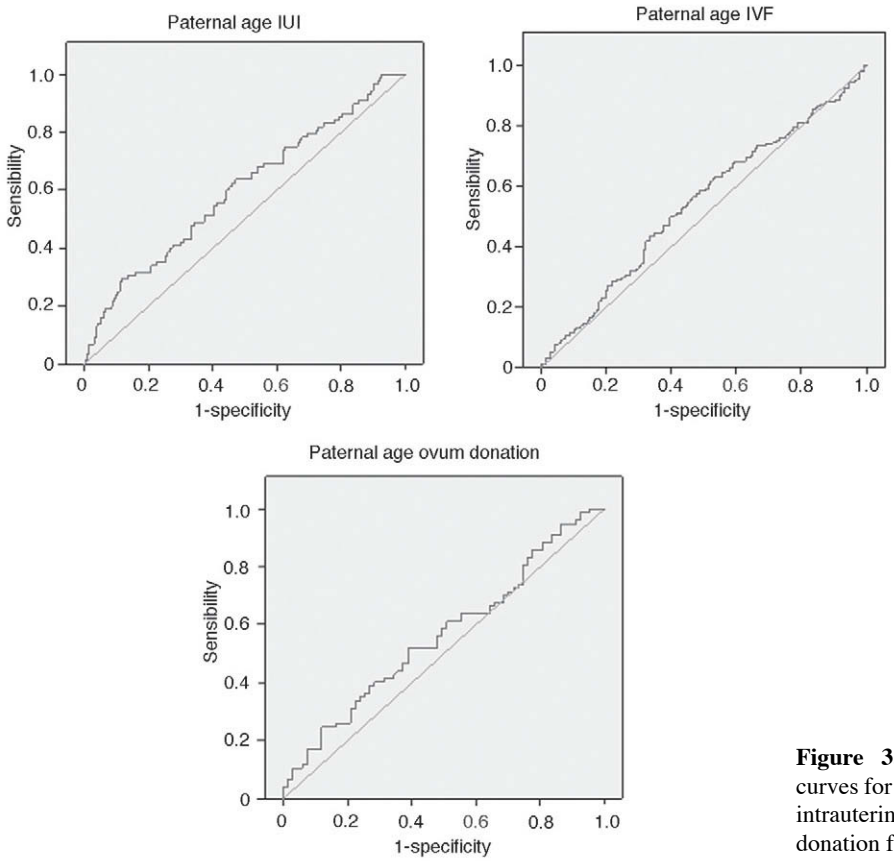

Figure 3. Receiver operating characteristic curves for the predictive value of paternal age in intrauterine insemination (IUI), IVF and ovum donation for the achievement of pregnancy.

Table 3. Logistic regression analysis of the effect of paternal age on pregnancy rates in intrauterine insemination (IUI), first cycles of IVF/intracytoplasmic sperm injection (ICSI) and first cycles of IVF/ICSI with donated oocytes.

Treatment	*Odds ratio*	*95% CI*	R^2 *Negelkerke*	P-*value*
IUI	1.056	0.990–1.128	0.036	0.014
IVF/ICSI	0.971	0.890–1.059	0.016	NS
Oocyte donation	1.019	1.000–1.039	0.040	0.048

CI = confidence interval; NS = not statistically significant.

Table 4. Correlations between paternal age and fertilization rate, embryo quality and implantation rate, in IVF/intracytoplasmic sperm injection (ICSI) and oocyte donation cycles.

Paternal age	*IVF/ICSI*		*Oocyte donation*	
	r	P-*value*	r	P-*value*
Blastomere number 48 h	0.031[a]	0.020	0.000	NS
Blastomere number 72 h	0.036[a]	0.020	0.001	NS
Embryo fragmentation 48 h	0.009[a]	NS	0.028[a]	0.013
Embryo fragmentation 72 h	0.010[a]	0.001	0.027[a]	0.016
Implantation rate	0.040	NS	0.036	NS
Fertilization rate	0.045	NS	0.056	NS

[a]Denotes a significant linear relationship, $P < 0.05$. NS = not statistically significant.

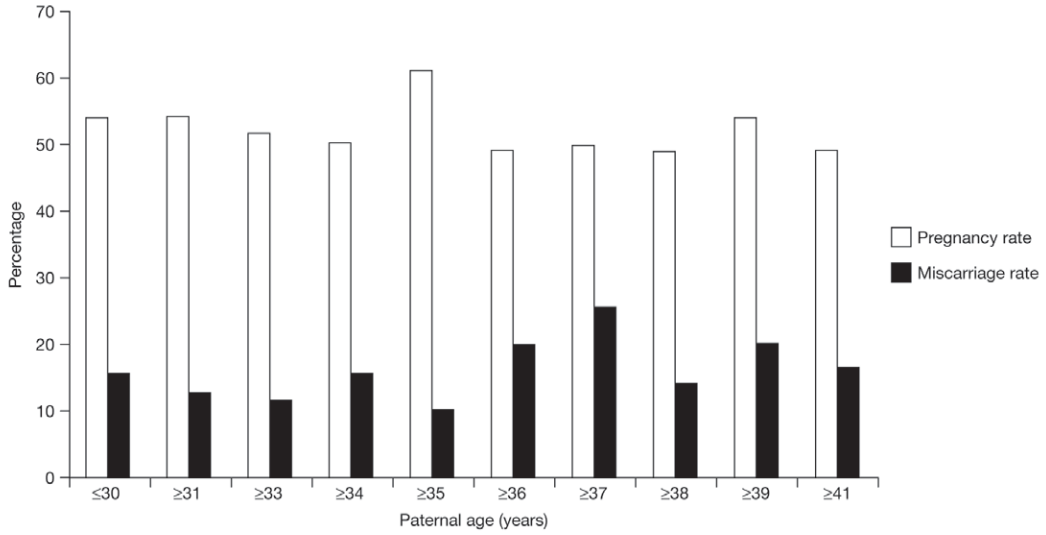

Figure 4. Pregnancy and miscarriage rates in IVF/intracytoplasmic sperm injection (ICSI) cycles according to male age.

Table 5. Couples undergoing IVF/intracytoplasmic sperm injection (ICSI) were categorized according to male age; the pregnancy results were then stratified according to female age.

Female age (years)	*Male age*									
	≤30	*≥31*	*≥33*	*≥34*	*≥35*	*≥36*	*≥37*	*≥38*	*≥39*	*≥41*
<30	54.6 (53/97)	65.9 (54/82)	48.8 (20/41)	50.0 (15/30)	66.7 (8/12)	40.0 (4/10)	100.0 (5/5)	37.5 (3/8)	50.0 (4/8)	46.7 (7/15)
31–35	52.3 (23/44)	47.0 (62/132)	54.9 (50/91)	52.7 (59/112)	61.2 (60/98)	47.0 (47/100)	50.9 (27/53)	59.0 (23/39)	58.8 (30/51)	43.4 (23/53)
36–38	50.0 (2/4)	57.1 (4/7)	75.0 (3/4)	26.7 (4/15)	55.0 (11/20)	60.0 (15/25)	40.5 (15/37)	37.0 (10/27)	47.1 (16/34)	59.4 (19/32)

Values in parentheses are number of pregnancies/number of patients.

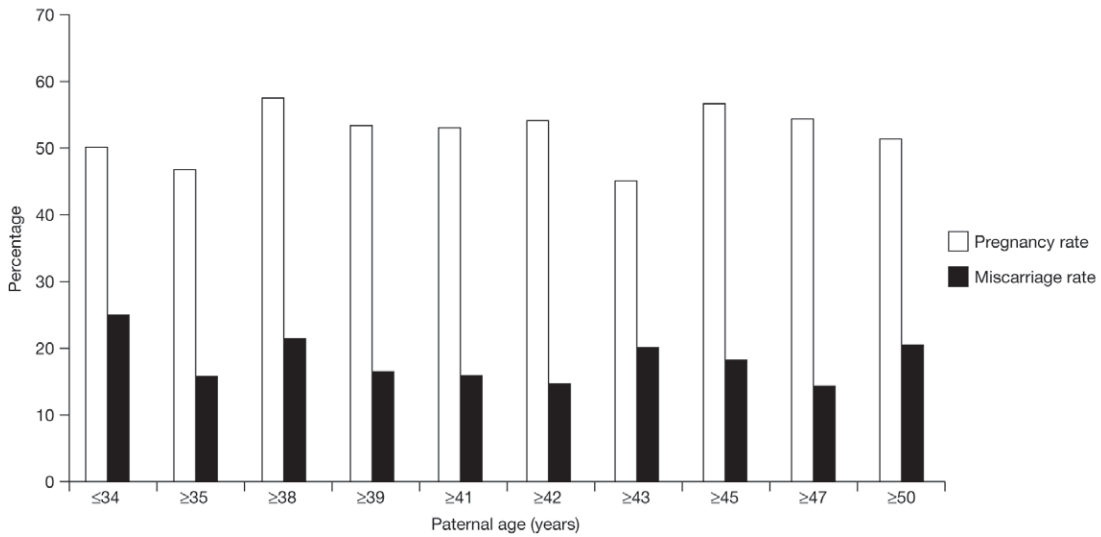

Figure 5. Pregnancy and miscarriage rates in ovum donation cycles according to male age.

involved could be impaired semen quality, maybe due to an increase incidence of urogenital infections, accumulation of toxic substances and anatomic and functional alteration of the seminal pathway (testis, epididymis and prostate) (Kuhnert and Nieschlag, 2004). Some reports have shown a continuous decrease in sperm motion and semen volume between 22 and 80 years of age, with no evidence of a threshold (Eskenazi *et al*., 2003; Sloter *et al*., 2006), as well as lower total sperm counts and sperm morphology (Klonoff-Cohen and Natarajan, 2004; Kuhnert and Nieschlag, 2004). In the present study, a significant negative association ($P < 0.05$) between volume and motility in fresh sperm and male age was also found (**Table 1**) when IUI, IVF and oocyte donation groups were considered separately or together (men from 19 to 71 years old).

Pregnancy chances after IUI (Mathieu *et al*., 1995) and IVF or GIFT (Klonoff-Cohen and Natarajan, 2004) have been shown to be lower with advanced male age. However, there is still controversy on this issue because relatively few children are born to older fathers (Kuhnert and Nieschlag, 2004) and scant studies have been performed regarding IUI and IVF outcome in advanced male age, some of them not finding a poorer prognosis (Spandorfer *et al*., 1998; Aboulghar *et al*., 2007). However, these reports have limitations due to the lower numbers of couples in the advanced age male category, and/or the confounding variable of the advanced age of the female partner.

Age-dependent decreases in fertility in couples are usually attributed to female ageing, and indeed the strong female age effect, and the fact that male and female age are related, make studies of male age effect on fertility difficult. The age-dependent increase of infertility, miscarriages, obstetric morbidities and chromosomal anomalies of the fetus in women is well documented (Kuhnert and Nieschlag, 2004). In order to avoid this bias, only young women (<38 years old) were considered. When IUI and IVF results were compared in the same range of maternal age (<30, 31–35, 36–38) among the different 10% width centiles of male age, no difference was found in terms of pregnancy rates (**Table 2** and **5**). Obviously, this evaluation was not performed in the oocyte donation group because all the egg donors are below 35 years by Spanish Law (Lacadena, 2006) and the mean age is 26 years old (Bellver *et al*., 2007), as in the present study.

However, few couples with very advanced male age were included. In fact, from the 4902 cycles included, only in 1046 (21.3%) of them were men older than 40 years (121 in IUI, 100 in IVF, and 825 in oocyte donation) and 109 (2.2%) older than 50 years (8 in IUI, 6 in IVF, and 125 in oocyte donation). Hence, the results can only be applied to men in the age range considered. Nevertheless, this is the age range that is usually found in infertility clinics.

So far as is known, no previous work has only considered first IVF cycles and two have been performed using the oocyte donation model (Gallardo *et al*., 1996; Paulson *et al*., 2001b). Only one previous report (Mathieu *et al*., 1995) assessed the influence of husband's age on the cumulative conception rate following IUI. They included 901 cycles, with a mean male age of 33.7 (range: 23–57). They concluded that cumulative pregnancy rate was higher when male age was below 30 years old (51.7% at five cycles), decreasing between 30 and 34 years old, and even more in men over 35 (25% at five cycles). No influence of maternal age was clearly seen, although women up to 43 years were included. In addition, couples with a long duration of infertility (up to 23 years), doubtful indications for IUI (abnormal Fallopian tubes, endometriosis) and different protocols for ovarian stimulation (clomiphene citrate, HMG and FSH) were considered. One or two inseminations per cycle were performed. In the IUI group, 2204 cycles were included, with a mean male age of 34.3 years (range: 25–56) and miscarriage rate of 21.5%. The cumulative pregnancy rate after three cycles was 44.4%, and 52.8% after four cycles. Only mild to moderate sperm factor and idiopathic infertility were included as indications for IUI, and rFSH was used for ovarian stimulation in all the patients. Two inseminations were always carried out in each cycle. Therefore, the study did not include the confounding variables present in the study by Mathieu *et al*. (1995). Pregnancy rates were similar among male age groups, when stratified (**Table 2**) or not (**Figure 1**) for maternal age. After a maximum of four IUI cycles, the achieved cumulative pregnancy rate did not vary according to male age (**Figure 2**). A logistic regression analysis showed a significant model but the 95% CI of the odds ratio included 1, and in consequence, paternal age was not a good predictive value for pregnancy outcome (**Table 3**). Thus, although a slight impact of male age appeared in IUI outcome, this is probably not clinically important.

Previous studies have shown a deleterious effect of paternal age on IVF (Klonoff-Cohen and Natarajan, 2004; de la Rochebrochard *et al*., 2006) but not in ICSI (Spandorfer *et al*., 1998; Aboulghar *et al*., 2007) outcome. In the study of de La Rochebrochard *et al*. (2006) (n = 1938 IVF cycles) men ≥40 years old presented a poorer IVF outcome but only when the female partner was ≥35 years old. Below this age, no negative effect was seen. Klonoff-Cohen and Natarajan (2004) (n = 221 IVF or GIFT/zygote intra-Fallopian transfer cycles) showed that each additional year of paternal age was associated with 11% increased odds of not achieving a pregnancy, and 12% odds of not having a successful live birth. They considered men from 22 to 55 years (mean 38.4 ± 5.68 years) and found a deleterious effect from 35 years of age, and especially from 40 years old, in all maternal ages. However, when maternal (continuous variable) and paternal age (potential confounder) were combined in the same statistical model, no significant impact of paternal age was detected on pregnancy and live birth delivery rates. Spandorfer *et al*. (1998) (n = 821 ICSI cycles) did not find differences in the ICSI outcome (implantation, pregnancy, miscarriage, ectopic pregnancy and live birth) or in laboratory parameters, based on paternal age. In this study, only the advanced maternal age was clinically relevant. Similarly, a recent study by Aboulghar *et al*. (2007) showed no differences in pregnancy rates. In the IVF/ICSI cycles (n = 1286), pregnancy and miscarriage rates were comparable among male age groups (**Figure 4** and **Table 5**), and also when female aetiology, female age and fertilization procedure (IVF, ICSI or both) were considered (data not shown). Multiple pregnancy rates as well as laboratory parameters (fertilization rate, embryo division and symmetry) did not vary, or varied only very slightly among male age groups (**Table 4**), but without any impact on the outcome (implantation, pregnancy and miscarriage rates).

Studies on IVF–embryo transfer cycles are biased by the wide heterogeneity of the female factor. In consequence, it is difficult to separate the potential deleterious effects of spermatozoa from those within the oocyte. To overcome these difficulties, ovum donation offers a powerful tool in the study of sperm effects. In the oocyte donation couples, the male age range was higher than in the IVF and IUI groups. Therefore, this group would give the more valuable information. Pregnancy and miscarriage rates did not vary among male age groups (**Figure 5**). Fertilization rates were similar as well as the number of blastomeres at 48 and 72 h of embryo development (**Table 4**). Only embryo fragmentation was slightly increased with advanced male age, but *r* values were not clinically relevant (similar implantation, pregnancy and miscarriage rates). By logistic regression analysis the variations in pregnancy rates for oocyte donation cycles induced by male age were significant ($P = 0.048$); nevertheless, the 95% CI of the OR included 1.0 and R^2 value was very low (0.04) (**Table 3**), and therefore the model presented is not clinically relevant. Only two previous works (Gallardo *et al*., 1996; Paulson *et al*., 2001a), with 345 and 558 oocyte donation cycles respectively, have analysed the influence of paternal age in the oocyte donation model. They found no differences in fertilization, pregnancy, implantation and live birth rates and also in in-vitro embryo development according to male age. In the three studied populations (IUI, IVF and oocyte donation), ROC curves showed a very low AUC value (near 0.5) of male age on the assisted conception outcome (**Figure 3**). This means that there is a lack of predictive value of male age (in the range considered) on assisted conception.

According to the present results, male age, in the range studied, slightly impairs semen quality but this effect does not lead to a poorer outcome in assisted conception when the female partner is not of advanced age. Therefore, male age should not be used as a prognostic factor in assisted reproduction. More studies are needed to evaluate the effect of extremely advanced paternal age on the reproductive outcome. However, recruiting such patients is very difficult due to their low demand for assisted conception and the frequently associated advanced age of the female partner. More studies performed in the oocyte donation model could help us to clarify this issue.

References

Aboulghar M, Mansour R, Al-Inany H *et al*. 2007 Paternal age and outcome of intracytoplasmic sperm injection. *Reproductive Biomedicine Online* **14**, 588–592.

Astolfi P, De Pasquale A and Zonta LA. 2006 Paternal age and preterm birth in Italy, 1990 to 1998. *Epidemiology* **17**, 218–221.

Auger J , Jouannet P 2005 Age and male fertility: biological factors. *Revue d'epidemiologie et de sante publique* **53** (Spec. No. 2), 2S25–35.

Bellver J, Melo MA, Bosch E *et al*. 2007 Obesity and poor reproductive outcome: the potential role of the endometrium. *Fertility and Sterility* **88**, 446-451.

Cardwell CR, Carson DJ, Patterson CC. 2005 Parental age at delivery, birth order, birth weight and gestational age are associated with the risk of childhood type 1 diabetes: a UK regional retrospective cohort study. *Diabetic Medicine* **22**, 200–206.

Choi JY, Lee KM, Park SK *et al*. 2005 Association of paternal age at birth and the risk of breast cancer in offspring: a case control study. *BMC Cancer* **5**, 143.

de la Rochebrochard E, de Mouzon J, Thepot F *et al*., 2006 Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France. *Fertility and Sterility* **85**, 1420–1424.

Eskenazi B, Wyrobek AJ, Sloter E *et al*. 2003 The association of age and semen quality in healthy men. *Human Reproduction* **18**, 447–454.

Fisch H, Hyun G, Golden R *et al*. 2003 The influence of paternal age on Down syndrome. *Journal of Urology* **169**, 2275–2278.

Gallardo E, Simon C, Levy M *et al*. 1996 Effect of age on sperm fertility potential: oocyte donation as a model. *Fertility and Sterility* **66**, 260–264.

Gourbin C. 2005 Foetal mortality, infant mortality, and age of parents. An overview. *Revue d'epidemiologie et de sante publique* **53** (Spec. No. 2), 2S81–6.

Harlap S, Paltiel O, Deutsch L *et al*. 2002 Paternal age and preeclampsia. *Epidemiology* **13**, 660–667.

Kleinhaus K, Perrin M, Friedlander Y *et al*. 2006 Paternal age and spontaneous abortion. *Obstetrics and Gynecology* **108**, 369–377.

Klonoff-Cohen HS, Natarajan L 2004 The effect of advancing paternal age on pregnancy and live birth rates in couples undergoing in vitro fertilization or gamete intrafallopian transfer. *American Journal of Obstetrics and Gynecology* **191**, 507–514.

Kuhnert B, Nieschlag E 2004a Reproductive functions of the ageing male. *Human Reproduction Update* **10**, 327–339.

Lacadena JR 2006 The 14/2006 law on human assisted reproduction techniques: scientific and ethical considerations. *Law Human Genome Review* **24**, 157–184.

Mathieu C, Ecochard R, Bied V *et al*. 1995 Cumulative conception rate following intrauterine artificial insemination with husband's spermatozoa: influence of husband's age. *Human Reproduction* **10**, 1090–1097.

Meseguer M, Molina N, Garcia-Velasco JA *et al*. 2006 Sperm cryopreservation in oncological patients: a 14-year follow-up study. *Fertility and Sterility* **85**, 640–645.

Meseguer M, Garrido N, Martinez-Conejero JA *et al*. 2004 Role of cholesterol, calcium, and mitochondrial activity in the susceptibility for cryodamage after a cycle of freezing and thawing. *Fertility and Sterility* **81**, 588–594.

Muriel L, Meseguer M, Fernandez JL *et al*. 2006a Value of the sperm chromatin dispersion test in predicting pregnancy outcome in intrauterine insemination: a blind prospective study. *Human Reproduction* **21**, 738–744.

Muriel L, Garrido N, Fernandez JL *et al*. 2006b Value of the sperm deoxyribonucleic acid fragmentation level, as measured by the sperm chromatin dispersion test, in the outcome of in vitro fertilization and intracytoplasmic sperm injection. *Fertility and Sterility* **85**, 371–383.

Paulson RJ, Milligan RC, Sokol RZ 2001 The lack of influence of age on male fertility. *American Journal of Obstetrics and Gynecology* **184**, 818–822; discussion 822–824.

Reichenberg A, Gross R, Weiser M *et al*. 2006 Advancing paternal age and autism. *Archives of General Psychiatry* **63**, 1026–1032.

Reichman NE, Teitler JO 2006 Paternal age as a risk factor for low birthweight. *American Journal of Public Health* **96**, 862–866.

Sartorelli EM, Mazzucatto LF, de Pina-Neto JM 2001 Effect of paternal age on human sperm chromosomes. *Fertility and Sterility* **76**, 1119–1123.

Slama R, Bouyer J, Windham G *et al*. 2005 Influence of paternal age on the risk of spontaneous abortion. *American Journal of Epidemiology* **161**, 816–823.

Sloter E, Schmid TE, Marchetti F *et al*. 2006 Quantitative effects of male age on sperm motion. *Human Reproduction* **21**, 2868–2875.

Spandorfer SD, Avrech OM, Colombero LT *et al*. 1998 Effect of parental age on fertilization and pregnancy characteristics in couples treated by intracytoplasmic sperm injection. *Human Reproduction* **13**, 334–338.

Tang CH, Wu MP, Liu JT *et al*. 2006 Delayed parenthood and the risk of cesarean delivery – is paternal age an independent risk

factor? *Birth* **33**, 18–26.
Tsuchiya KJ, Takagai S, Kawai M *et al*. 2005 Advanced paternal age associated with an elevated risk for schizophrenia in offspring in a Japanese population. *Schizophrenia Research* **76**, 337–342.
Vestergaard M, Mork A, Madsen KM *et al*. 2005 Paternal age and epilepsy in the offspring. *European Journal of Epidemiology* **20**, 1003–1005.
Zhu JL, Madsen KM, Vestergaard M *et al*. 2005 Paternal age and congenital malformations. *Human Reproduction* **20**, 3173–3177.

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