ASSISTED REPRODUCTION

Effects of men and recipients' age on the reproductive outcome of an oocyte donation program

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Abstract
Purpose The objective of this study was to evaluate the effect of men and recipient age on the reproductive outcome of our oocyte donation program.
Methods We retrospectively analyzed 915 cycles, taking into account men and recipient age, separately and together.

Capsule Aging could have a detrimental effect on the reproductive outcome of an oocyte donation program when recipient and men age are higher than 38 year.

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Results The significant cut off value for men and recipients age with incidence in the reproductive outcome was 39 years. Recipient older than 38 years presented a significantly lower pregnancy and implantation rates than others (44.92 vs. 55.75±1.53%, 25.66 vs. 32.79±1.64%). If men age was older than 38, a significant reduction in pregnancy and implantation rates was observed, too (46.0 vs. 54.65%, 26.00±1.52 vs. 32.43±1.65%). When men and recipient age was analyzed together, a reduction in pregnancy and implantation was detected only if both were older than 38.
Conclusions Present study suggests that age has a detrimental effect on the reproductive outcome of oocyte donation cycles when both men and recipient are ≥ 39 years old.

Introduction

In the last two decades there has been a great increase in childbearing among women of mature age in the industrialized world [1]. This is due in part to planned delayed childbearing, but a major contributor is the use of assisted reproductive technology (ART) in women of relatively advanced maternal age [2]. It is well known that maternal age adversely affects fecundity; reproductive capacity in women declines dramatically after they reach 40 [3] because of an abrupt loss of functional oocytes, increasing risk of chromosomes defects, spontaneous abortion, etc. [4]. However, the huge advances in reproductive medicine have compensated, to some extent, for this natural decrease in fecundity, and older women can become pregnant through oocyte donation [5].

Until the last few years little attention has been paid to the possible effects of paternal age in the reduction of

fecundity. Advanced men age has been associated with a significant reduction in pregnancy rates, increased time-to-pregnancy and increased sub-fecundity [6, 7]. Some recent papers relate men age with risk of increased DNA damage [8], spontaneous abortion [9], stillbirth [10], fetal death [11] and birth defects [12].

The question of age-related changes in semen quality remains unsolved. The weight of evidence from clinical [7] and healthy men studies [13] suggests that age is associated with diminished semen volume, sperm motility and/or morphology, but sperm concentration is affected little by age [14–16]. However, other authors noted no change in sperm quality [17].

The oocyte donation provides a powerful tool to analyze the influence of men aging on reproductive potential because in these patients oocytes are obtained from a homogeneous and young population of oocyte donors, reducing the variability associated to the oocyte quality from patients.

The purpose of this paper was to evaluate the effect of man and woman age on the reproductive outcome of our oocyte donation program, and study if there is an interaction between man and recipient age.

Materials and methods

This retrospective study comprises 915 oocyte donation cycles from 662 couples (each couple have performed 1.38 cycles on average), performed at the Instituto Valenciano de Infertilidad in Murcia and Almeria from January 1996 to December 2006. In these couples, there was no severe male factor infertility. The following parameters were recorded and evaluated for each cycle: donor, recipient and man age on the day of the oocyte retrieval, semen analysis the same day including volume, sperm concentration, total sperm count in the ejaculate, motile (A+B grade) rate, total motile sperm in the ejaculate and normal sperm rate, fertilization rate, the number of blastomers and fragmentation rate at the embryo transfer day, number of transferred embryos, and pregnancy, implantation and miscarriage rates. Each donation cycle was treated as an independent event.

Oocyte donors

All donors were included in the oocyte donation program after being thoroughly informed and having fulfilled the inclusion criteria. All of them were aged between 18 and 35, with a mean age of 25.03 ± 0.15. We had access to their complete medical history, which included current or past exposure to radiation or hazardous chemical substances, iv. drug use, and reproductive history. All subjects were shown to be normal in a physical and gynecological examination, had no family history of hereditary or chromosomal

diseases, had a normal karyotype, and tested negative in a screening for sexually transmitted diseases.

The protocol for ovarian stimulation has been described elsewhere [18]. In brief, all donors underwent a long protocol of down-regulation with daily doses of a GnRH agonist (GnRH-a). Trans-vaginal ultrasound was performed to ascertain ovarian quiescence on the first 3 days of menses, and controlled ovarian stimulation was then initiated. The starting dose varied from 150 to 300 U/d of FSH and/or human menopausal gonadotropin for the first 2–5 days, according to age, body mass index, and response to previous ovarian stimulations. Dose was then adjusted according to ovarian response, which was monitored through serum E_2 levels and ultrasound every 2–3 days. Stimulation was performed until leading follicles had a mean diameter of more than 18 mm. Human chorionic gonadotropin was then administered, and ovarian retrievel was performed 36 h later. Anonymous donors were matched with their recipient(s) according to physical characteristics and blood group.

Oocyte recipients

Oocyte recipients entered oocyte donation program due to one of the following diagnosis: premature ovarian failure/menopause, genetic or chromosomal disorders, low response to controlled ovarian hyper-stimulation, failure to achieve pregnancy after at least three cycles of assisted reproduction techniques, or recurrent miscarriage [5]. Recipients' mean age was 38.26 ± 0.17, ranging from 22 to 52 years old, and mens' mean age was 39.10 ± 0.20, ranging between 24 and 66 years old. Because excess weight could be a risk factor for spontaneous abortion in oocyte donation program [19], recipients' body mass index (BMI) was recorded.

Oocytes recipients underwent hormone replacement therapy, as previously described [18]. In patients with ovarian function, a depot GnRH-a was administered in the midluteal phase of their cycle. Hormone replacement was initiated on day 1–3 of the following cycle, and doses of estradiol valerate (EV; Progynova; Schering Spain, Madrid, Spain) were administered as follows: 2 mg/d for the first 8 days of treatment, 4 mg/d for the following 3 days, and 6 mg/d until a pregnancy test was performed after embryo transfer. On the 15th or 16th days of hormone replacement, a trans-vaginal ultrasound was performed to measure endometrial thickness, and serum E_2 levels were tested. Recipients without ovarian function were submitted to the same endometrial preparation protocol, with the exception of the administration of the depot GnRH-a. Micronized progesterone (800 mg/d, vaginally; Progeffik; Effik Laboratories, Madrid, Spain) was initiated the day after oocyte retrievel, and embryos were transferred 48 h or 72 h after aspiration.

Embryos were produced by IVF or ICSI, depending on semen characteristics on the day of the ovarian puncture. Embryos were classified on day 2 and 3 after oocyte retrieval according to cell number and degree of fragmentation [20]. The number of embryos to be replaced was decided based on embryo quality, patient's age, the outcomes of previous assisted reproduction treatments, reproductive history, and the presence of uterine malformations. The embryo transfer was performed under ultrasonographic guidance with a flexible intrauterine catheter (Entrac Delphin, Gynétics Medical Products N.V., Belgium).

Serum ß-human chorionic gonadotropin was measured 14 days after oocyte retrieval. Clinical pregnancy was confirmed 2 weeks later if the existence of a gestational sac was observed by transvaginal ultrasonography (US).

Semen analysis and processing

Semen samples were collected by masturbation after a period of sexual abstinence of 3 to 5 days. Samples were allowed to liquefy at room temperature before a semen analysis was performed according to standard World Health Organization criteria [21]. The following parameters were determined by standard assessment: volume, sperm concentration, total sperm in ejaculate, motility, total number of motile spermatozoa and normal morphology. Sperm morphology evaluation was performed according to strict criteria [22]. Semen samples were prepared in the IVF laboratory by swim-up or density gradients centrifugation.

Statistical analysis

Data are expressed as the mean $\pm$ SEM (standard error of the mean) and analyzed by ANOVA (analysis of variance), considering the men and recipient age as the main variables. When ANOVA revealed a significant effect, values were compared by the LSD (least significant difference test) post hoc test. Differences were considered statistically significant at $p<0.05$.

Categorical data (fecundation rate, mean number of blastomeres, mean rate of embryo fragmentation degree, pregnancy and implantation) were modeled according to the binomial model of parameters and were analyzed by ANOVA.

We calculated the pregnancy and implantation rates for the groups of men and recipients age between <32 and >45 years, and evaluated their differences between groups and the distribution of the cases in the proposed groups.

Results

A total of 915 cycles of oocyte donation with embryo transfers were analyzed. General parameters related to oocyte donors, recipients and men and data related to fertilization outcome, embryo transfer and pregnancy are presented in Table 1. These data were expected in our IVF lab.

Reproductive outcome and seminal parameters related to men's age

We selected 39 years as the cut-off value of men age according to the most equilibrated distribution of cases in both age groups ($n=452$ vs.463) and the maximum difference in pregnancy (8.65%, $p=0.01$) and implantation (9.67%, $p=0.01$) rates between groups, after we evaluated these parameters on groups of patients aged from <32 to >45 years.

The classification of men according this cut-off value of 39 years showed that when men were equal to or older than 39 years, the reproductive outcome, in terms of pregnancy (54.65 vs. 46.00 %, $p=0.01$) and implantation rates (32.43$\pm$1.65 vs. 26.00$\pm$1.52 %, $p<0.01$; Table 2), were significantly reduced. Donors age, recipients BMI, fertilization rate, embryo quality (number of blastomers and fragmentation degree), number of replaced embryos, number of embryonic sacs and miscarriage rate were similar in both groups of men age ($p>0.05$). Both men and recipient's age were significantly different between the two groups ($p<0.01$).

Semen quality from men grouped according to this age presented significantly higher forward motility (A+B grade) rate in younger men (44.35$\pm$0.93 vs. 37.36$\pm$1.02; $P<0.01$; Table 3), no other seminal parameters were different.

Reproductive outcome related to recipient's age

In the same way that we previously selected the best cut-off value for men age, we selected 39 as the best cut-off value for recipients age according to the maximum difference

Table 1 General parameters of 915 of oocyte donation cycles

Parameter	Mean $\pm$ sem	Range
Donors age (yr)	25.03$\pm$0.15	18–36
Recipients' age (yr)	38.26$\pm$0.17	22–52
Recipients' BMI	24.22$\pm$0.22	17.53–37.64
Men's age (yr)	39.10$\pm$0.20	21–61
Fertilization rate (%)	82.71$\pm$0.59	25–100
Mean number of blastomers	5.63$\pm$0.07	2–10
Mean rate of fragmentation (%)	10.45$\pm$0.29	0–58
Pregnancy rate (%)	50.27	
Implantation rate (%)	29.18$\pm$1.12	0–100
No. of embryonic sacs	1.48$\pm$0.03	1–4
Miscarriage rate (%)	20.43	

Table 2 Reproductive parameters from 915 cycles of oocyte donation program categorized by age in men younger and older than 38 years

Parameter	Men`s age < 39 years	Men's age ≥ 39 years	p-value
N	453	462	
Men's age (yr)	34.37±0.14	43.71±0.22	<0.01
Donors' age (yr)	25.23±0.22	24.83±0.20	0.18
Recipients' age (yr)	35.33±0.22	41.12±0.17	<0.01
Recipients' BMI	24.22±0.29	24.21±0.33	0.99
Fertilization rate (%)	83.61±0.80	81.88±0.85	0.14
Mean number of blastomers	5.53±0.09	5.73±0.09	0.12
Mean rate of fragmentation (%)	10.45±0.41	10.46±0.42	0.99
No. of replaced embryos	2.63±0.04	2.70±0.04	0.25
Pregnancy rate (%)	54.65%	46.00%	0.01
Embryo implantation rate (%)	32.43±1.65	26.00±1.52	<0.01
No. of embryonic sacs	1.49±0.04	1.46±0.04	0.66
Miscarriage rate (%)	20.24%	20.66%	0.91

between groups of age in pregnancy (10.83%; $p<0.01$) and implantation (7.13%; $p<0.01$) rates, and the most equilibrated distribution of the cases in both groups ($n=452$ vs.463).

With these groups of recipient's age we evaluated the reproductive performance and recipient younger than 39 years presented a higher pregnancy (55.75% vs. 44.92%, $p<0.01$) and implantation rates (32.79±1.64 vs. 25.66±1.53, $p<0.01$, Table 4). The other reproductive parameters were similar in both groups and only men and recipient's age were significantly different.

Reproductive outcome related to both recipient's and men's age

We observed that men and recipient age were always related, in general "young" men were with "young" recipient and "old" men with "old" recipient. In order to avoid this age correlation patients were divided into four groups: men and recipient younger than 39 years; men 39 or older and recipient younger than 39; men younger than 39 and recipient 39 or older; men and recipient 39 or older. Only when both members were "old", we observed that age negatively affected pregnancy and implantation rates. When

one or the two members of the couple were "young" no age effects were observed (Table 5).

These results are based on 915 cycles of oocyte donation from 662 couples, that means each couple have performed 1.38 cycles on average, and so the cycles analyzed were not completely independent. In order to determine if that could influence our results, data of only one cycle per couple were considered. The same tendency was found when 662 cycles were analyzed.

Discussion

Oocyte donation provides a good model to examine the effect of ageing men and/or women on fertility because such oocytes are obtained from a homogeneous and young population of donors. This fact reduces the variability associated to the oocyte quality, which is higher in own patients' oocytes, and only uterus receptivity and sperm quality are involved in reproductive success. The objective of this study was to investigate the influence of men and recipient age on the reproductive outcome of this group of treatment.

The results from our preliminary analysis to select the cut-off value of 39 years in recipient are in agreement with

Table 3 Seminal parameters from 915 cycles of oocyte donation program categorized by age in men younger and older than 38 years

Seminal parameters	Men's age < 39 years	Men's age ≥ 39 years	p-value
Volume (ml)	2.74±0.09	2.56±0.09	0.17
Sperm concentration (10^6 cells/ml)	46.54±1.71	49.36±1.89	0.27
Total sperm in ejaculate (10^6 cells)	120.05±5.60	123.82±6.63	0.66
Motility A+B (%)	44.35±0.93	37.36±1.02	<0.01
Total motile sperm in ejaculate (10^6 cells)	61.74±3.45	57.59±3.69	0.41
Normal Morphology (%)	12.42±0.60	13.15±0.76	0.45

Table 4 Reproductive parameters from 915 cycles of oocyte donation program categorized by age in recipient younger and older than 38 years

Parameter	Recipient age < 39 years	Recipient age ≥ 39 years	p-value
N	456	459	
Recipients' age (yr)	34.04±0.15	42.37±0.12	<0.01
Donors' age (yr)	25.28±0.22	24.78±0.20	0.09
Recipients' BMI	24.14±0.33	24.29±0.28	0.73
Men's age (yr)	35.81±0.20	42.31±0.28	<0.01
Fertilization rate (%)	83.17±0.84	82.26±0.82	0.44
Mean number of blastomers	5.54±0.09	5.72±0.09	0.17
Mean rate of fragmentation (%)	10.21±0.40	10.70±0.43	0.41
No. of replaced embryos	2.66±0.04	2.67±0.04	0.86
Pregnancy rate (%)	55.75	44.92	<0.01
Embryo implantation rate (%)	32.79±1.64	25.66±1.53	<0.01
No. of embryonic sacs	1.49±0.04	1.47±0.05	0.75
Miscarriage rate (%)	21.03	19.71	0.73

some previous data suggesting a decrease in fertility around the age forty in recipient [4]. Studying egg donation in recipient of different ages sharing oocytes from the same donor, Borini et al. [23] observed that pregnancy and implantation rates were higher in recipient under the age of 40 compared to those above the age of 40 and concluded that this difference was due to uterine factors. No significant difference in the miscarriage rate between the two groups was reported. An evaluation of the role of recipient age on the outcome of donor egg cycles in the United Stated [24] in a period of 3 years showed no effect of recipient age between ages 25 and 45 years. Older recipient age, however, was associated with statistically reduced implantation, clinical pregnancy and delivery rates. This effect first appeared among recipients in their late 40s,

and became more pronounced at age >50 years. Soares et al. [18] reported that in donated oocyte recipients pregnancy and implantation rates were significantly reduced and miscarriage rate was significantly increased from 45 year of age onward. Cano et al. [25] found that both pregnancy and implantation rates were not significantly different between young or old recipients. However, they reported a significantly higher miscarriage rate in recipient above the age of 40. They suggested that the mechanisms responsible for placental formation and function in the uterus could be affected by age.

Other authors found opposite results. Navot et al. [26] performed a prospective trial in which they showed that the age of the uterus did not affect the outcome of pregnancy and miscarriage when oocytes were taken from the same

Table 5 Reproductive outcome of the 915 cycles of oocyte donation cycles in age groups

	Recipients' <39 years		Recipients' >39 years		
	Men's < 39 years	Men's > 39 years	Men's < 39 years	Men's > 39 years	p-value
n	338	114	114	349	
Men's age (yr)	33.99[a]	41.19[b]	35.50[c]	44.53[d]	<0.01
Recipients age (yr)	33.26[a]	36.37[b]	41.46[c]	42.67[d]	<0.01
Recipients BMI	24.30	23.48	24.02	24.41	0.67
Donors age (yr)	25.49	24.67	24.47	24.89	0.10
Fertilization rate (%)	83.34±0.94	82.90±1.81	84.39±1.55	81.55±0.97	0.40
Blastomers	5.41[a]	5.92[b]	5.87[ab]	5.67[ab]	0.04
Fragmentation rate (%)	10.38	9.70	10.66	10.71	0.75
Embryos replaced	2.69[ab]	2.60[ab]	2.48[a]	2.74[b]	0.05
Pregnancy rate (%)	56.21[a]	54.39[a]	50[a]	43.27[b]	0.01
Implantation rate (%)	32.93±1.89[a]	32.37±3.28[a]	30.96±3.32[ab]	23.93±1.70[b]	<0.01
Embryonic sacs	1.50	1.44	1.46	1.47	0.93
Miscarriage rate (%)	20.53	22.58	19.30	19.87	0.97

[a,b] Numbers within rows with different superscripts differ (p<0.05)

donor and randomly allocated to recipients below and above the age of 40. They concluded that capacity to conceive and maintained a gestation when oocyte quality is controlled appears to be independent of uterine age. Using the same model, Abdalla et al. [27] reported similar pregnancy and miscarriage rate when recipients were younger or older than 39 years, concluding that decline in fertility with age can not be explained by uterine factors alone. Noyes et al [28] found, using five age groups (<35, 35–39, 40–44, >45), that recipient age does not adversely affect pregnancy potential. In a large group of unselected patients, Budak et al. [5] did not detect statistically significant difference in pregnancy and cumulative pregnancy rates in advanced-age recipient (>40 years) compared with younger recipients.

The cut-off value of 39 years obtained for men was less predictable than in recipient, because the information about the effect of ageing men on reproductive outcome is scarcer, and controversial results have been reported [5, 6]. In line with the present study, Paulson et al. [29] investigated the effect of men aging on sperm quality as determined by semen analysis, fertilization rate in vitro, and live birth rate in an oocyte donation model. However, no effect of men age on sperm fertilizing potential, sperm function, or pregnancy outcome was detected. As it has been suggested, in studies examining fertility status of ageing men, the results are most likely confounded by female partner age [7], and this could have been the reason for the lack of influence of age reported in Paulson's study.

As Klonoff-Cohen and Natarajan [30] found in *in vitro* fertilization or gamete intrafallopian transfer, we observed a decrease in reproductive success with aging men. In order to not confound the results by recipient age we divided our oocyte donation cycles in four different groups of age: men and recipient "old", men and recipient "young", men "young" and recipient "old", men "old" and recipient "young". Then we only observed difference in pregnancy and implantation rate when men and recipient were over 39.

Recent studies have demonstrated an increase in sperm DNA damage with age in healthy [8, 31, 32] and infertile men [33–35]. DNA fragmentation has been proposed as one of the reasons for the negative paternal effects on reproductive outcome, because a high percentage of DNA fragmentation has been correlated with low pregnancy rate [36]. As we have found with men age, no association between DNA fragmentation in spermatozoa and fertilization rates in patients undergoing ART (IVF and ICSI) was found [23, 33, 36]. However, a negative relation between the DNA damage in spermatozoa and blastocyst development after IVF and ICSI was observed in unprocessed spermatozoa [37] and in processed spermatozoa for IVF [38]. Frattareli et al [39] reported in donor oocyte assisted reproductive technology cycles a decrease in blastocyst formation rate

when men were >50 years of age. This correlation has not been detected when day 2 and day 3 embryo development was assayed. This was expected because the maternal genome mainly controls the first embryo divisions, and embryonic genome start to control embryo development after the 8 cells stage [40]. Our data on embryo development are in agreement with findings of other studies evaluating paternal age using the donor oocyte model [17, 29]. As we have transferred day 2 or day 3 embryos, we do not have information on blastocyst development in our patients, but a hampered embryonic development caused by DNA fragmentation could produce a decrease in pregnancy and implantation rates.

In the present study, only when men and recipient were older than 38 years, we found that age had a negative effect on the reproductive outcome of our oocyte donation program (pregnancy and implantation rates). In a study where the effects of men and recipient age on natural fertility were evaluated [41], similar results were exhibited. Fertility (measured by the probability of clinical pregnancy after intercourse on a given day relative to ovulation) decreased with paternal age, but only among couples composed of a woman aged 35 to 39 years. de La Rochebrochard et al. [42] in a retrospective, population-based sample, including fifty nine French IVF centres reported that men older than 39 years presented more difficulties in having a baby than younger men, when their female couple ages were increased.

It is widely accepted that implantation of the human embryo, defined as the process by which the embryo attaches to the endometrium, invades the decidualized stroma and reaches the maternal microvasculature, is a complex phenomenon that includes a large variety of biochemical and biophysical progressive modifications leading to maternal-embryonic interaction [43]. Besides, there is an embryonic-maternal dialogue, in which the embryo and the maternal reproductive tract induce changes in each other to promote embryonic development and endometrial receptivity. Advanced men age might produce embryos of inferior quality than those of young men because of the DNA fragmentation. Moreover, advanced age women could produce inferior endometrium than young ones, so those low quality embryos would need a "perfect" endometrial environment to implant, and that is not the case in "old recipient". If decidual quality is optimum those embryos could implant more easily.

Regarding the results about sperm motility, our findings agree with those from Eskenazi et al. [13], since these authors reported a reduction in sperm motility in men older than 39 years. However, contradictory findings about the effect of men age on seminal parameters have been reported: A recent study showed that semen volume and total motility decreased with increasing men age [39]. A

meta-analysis of male fertility published by Kidd et al [7] concluded that increasing age is associated with decreased semen volume, sperm motility, and number of morphologically normal sperm. The strongest decreases were observed for comparison of 30-year-old men with 50-year-old men. Spandorfer et al [44] found a significant linear decline in semen volume, but no significant differences in the concentration, motility or morphology of the spermatozoa were detected with paternal ageing. Finally, Gallardo et al [17] reported similar sperm characteristics among men of different ages in an oocyte donation program. It seems then, from the data in the bibliography, that sperm motility is the most frequent parameter undergoing alterations as the men age increases, and precisely this was the only significant difference observed in the present data. An explanation to age related motility decrease has been recently proposed [45]. Age-dependent changes in sperm motility and other motility-related parameters are related to flagellar zinc content. According to these authors, removal of zinc from the outer dense fibers during epididymal sperm maturation is affected in aging men, which in turn will result in decreased sperm motility. Although zinc concentration in the seminal fluid, flagella, or the whole spermatozoa was not measured in the present study, the above proposed explanation could fit with our data.

In conclusion, the results of this study demonstrate for the first time that there exits a relationship between men and recipient age with the reproductive outcome of oocyte donation cycles. When both men and recipient are 39 years or older, there is a detrimental effect on pregnancy and implantation rates. This information could be useful for couples postponing childbearing beyond their late thirties.

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