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Fathers over 40 and increased failure to conceive: the lessons of in vitro fertilization in France

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FIVNAT is an association which was founded in 1986. Most French IVF centres belong to the FIVNAT association. The FIVNAT association is directed by a committee elected every two years. The current committee was elected in September 2004 and is composed of Philippe ARVIS, Jean-Philippe AYEL, Joëlle BELAISCH-ALLART, Jacques CHOUTEAU, Laurent JANNY, Rachel LEVY, François MOUCHEL, Jean-Luc POULY (chairman), Dominique ROYERE, Jean-Paul TAAR. The FIVNAT association is principally funded by Organon Pharmaceuticals Inc.

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Running title: Fathers over 40 and failure to conceive

Capsule. As an increasing number of couples choose to postpone childbearing, they should be informed that paternal age over 40 years is an important risk factor for failure to conceive.

ABSTRACT

Objective: To investigate paternal age effect mediated by biological modifications with use of data from assisted reproductive technologies.

Design: National IVF registry.

Setting: France.

Patients: 1,938 men whose partners were totally sterile, with bilateral tubal obstruction or absence of both tubes (in order to avoid bias sampling in analysis of paternal age) and treated by conventional IVF.

Intervention: None.

Main outcome measure(s): Risk of failure to conceive defined as absence of intrauterine pregnancy.

Results: The odds ratio of failure to conceive for paternal age ≥40 years was 2.00 (95% CI: 1.10-3.61) when the woman was aged 35-37 years, 2.03 (95% CI: 1.12-3.68) for age 38-40 years, and 5.74 (95% CI: 2.16, 15.23) for age 41 years and over.

Conclusions: As an increasing number of couples choose to postpone childbearing, they should be informed that paternal age over 40 years is an important risk factor for failure to conceive.

Key words. Paternal age; Maternal age; Infertility; Fertilization in Vitro

INTRODUCTION

In industrialized countries, demographers have observed a trend to delay childbearing, reflecting couples' desire to have children at older ages. However, the risk of reproductive difficulties is clearly increased for couples who delay childbearing until after the age of 35. Maternal age over 35 years increases risks of infertility, miscarriage and ectopic pregnancy (1, 2). Moreover, a recent simulation model showed that assisted reproductive techniques (ART) "do not fully compensate for the years (and the chances of conceiving) lost" (3). This marked maternal age effect led to the conclusion that 35 years is the "amber light" in the reproductive life of women (4).

Paternal age was long almost ignored in studies of age effect on reproductive outcomes, but its potential role has recently been investigated. Some works have shown that increasing paternal age is accompanied by greater risk of delay in achieving pregnancy, of miscarriage and of late fetal death (5-8). In a recent review of the literature, we considered that 40 years could be the "amber light" in male reproductive life, as is 35 years for women's reproductive life (9). The demonstrated effect of paternal age on risk of delay in achieving pregnancy could be the consequence of either biological modification of the male reproductive tract or of decrease in male sexual activity. When analyzing natural conception, it is very difficult to distinguish sexual and biological consequences of age. In order to analyze paternal age effect mediated by biological aging alone, data on medically assisted cycles provide a very interesting model.

Data on medically assisted reproduction have been used to confirm a biological effect of maternal age on the probability of conception (10). In order to avoid sampling bias in analysis of infertile couples, Schwartz *et al.* selected couples

requesting artificial insemination with donor semen (AID) because the men were totally sterile (azoospermic men only). Among these couples whose sterility was linked to male reproductive impairment, the authors hypothesized that the women's fecundity was comparable to that of the general population, and so the maternal age effect in this population requiring AID could be extrapolated to the general population. This study confirmed that maternal age affected the probability of conception, mediated by biological aging of the women. It showed that this effect began as early as 30 years and became significant after age of 35. To confirm a biological effect of paternal age, the methodology of Schwartz *et al.* could be applied by selecting couples requiring medical assistance because the wife was totally sterile.

In order to confirm the hypothesis of a biological paternal age effect on the risk of failure to conceive, we studied ART data from the French national in vitro fertilization (IVF) registry by selecting couples requesting IVF because the woman was totally sterile, that is to say with bilateral tubal obstruction or absence of both tubes.

MATERIALS AND METHODS

Since 1986, the French National IVF Registry (FIVNAT) has collected information on aspiration cycles carried out in France (11). The FIVNAT registry received approval from the French Data Protection Authority (CNIL) on 17 December 1987 (declaration n° 174 168). IVF centers participate voluntarily in this registry. The 79 centers currently belonging to FIVNAT perform nearly 90% of the aspiration cycles in this country. We carried out data quality control on FIVNAT centers concerning fulfilling of key items and thus restricted our analysis to 59 centers (59/79=75%). In order to analyze paternal age, we investigated couples requesting conventional IVF in which the female partners were totally sterile, i.e. with bilateral tubal obstruction or absence of both tubes. To avoid bias due to changes in ART techniques (especially related to increasingly widespread use of intracytoplasmic sperm injection), we restricted our investigation to IVF performed since 2000. Finally, 1,938 couples treated by conventional IVF for bilateral tubal obstruction were included in this study.

We analyzed the risk of failure to conceive, defined as absence of intrauterine pregnancy confirmed by echography and an HCG level >1000 IU. Age effect was considered by using five-year age classes. As the age of 37 years has previously been demonstrated to be a cut-off point for the effect of maternal age on IVF success rate (12), we divided the group of women aged 35–40 into two sub-groups, 35-37 years and 38-40 years.

Age effects were analyzed based on odds ratios estimated by logistic regression using the SAS system (v8.02) package. Estimation of odds ratios relies on the method of maximum likelihood and confidence intervals for odds ratios were computed based on individual Wald tests. In a first logistic multivariate model, we

analyzed paternal age effect by controlling for maternal age effect. This model is based on the hypothesis that the paternal age effect is the same whether the woman is young, middle-aged or older. This hypothesis has been debated in some studies which indicated that the paternal age effect may be greater when the woman is aged 35 years and over than among younger women (7, 8). In order to take into account the possibility that paternal age effect may differ according to maternal age, we also used a second model which included an interaction factor between maternal age and paternal age.

RESULTS

As shown in table 1 and in table 2, the risk of failure to conceive clearly increased with maternal age and with paternal age in both models. In table 1, without male and female age interaction, a significant maternal age effect appeared in women aged 38-40 years and in women aged ≥41 years. The odds ratio (OR) for paternal age ≥40 years compared to <30 years was 1.70 (95% CI: 1.14-2.52).

In table 2, taking into account an interaction between male and female ages, the odds ratio of failure to conceive for paternal age ≥40 years was 2.00 (95% CI: 1.10-3.61) when the woman was aged 35-37 years, 2.03 (95% CI: 1.12-3.68) for age 38-40 years, and 5.74 (95% CI: 2.16, 15.23) for age 41 years and over.

DISCUSSION

Our results provide for the first time strong evidence for a paternal age effect on failure to conceive that is linked only to biological male aging (without confusion with sexual activity). We observed a clear tendency to increased risk of failure to conceive, especially when the fathers were aged over 40 years. Results in the first and last classes in table 2 (older woman with young man or young woman with older man) should be interpreted with caution because of the small number of couples in these classes. We thus analyzed table 2 by concentrating on classes with at least 30 couples. This revealed a clear increase in risk of failure to conceive with paternal age over 40 years when the woman was aged 35 years and over.

The paternal age effect was demonstrated here in a population of couples treated in IVF programmes and who were highly selected on the fertility characteristics of the woman (women who were totally sterile, that is to say with bilateral tubal obstruction or absence of both tubes). This finding can be extrapolated to the general population based on the hypothesis that these sterile women have no tendency to bond with men having any particular fertility characteristics. To the best of our knowledge, there is no biological or sociological evidence at the present time that could seriously question this hypothesis.

Our results on a paternal age effect after 40 years are in accordance with results recently published concerning the general population. In a European population-based study of couples attempting to conceive naturally, a significant odds ratio of 2.99 (95% CI: 2.77, 7.55) for risk of not having conceived after 12 months of attempting to achieve pregnancy was observed when the woman was aged 35-39 years and the man 40 years and over (7). A similar tendency was observed in another European study of 782 couples, which showed a decrease in the

daily probability of conception in couples composed of a woman aged 35-39 years and of a man in his late thirties or older (8).

It has been shown that couples having difficulty in conceiving also have an increased risk of miscarriage (19). Thus, the association between paternal age and failure to conceive raised the question of a possible association between paternal age and miscarriage. In the literature, an increased risk of miscarriage was observed in couples composed of a woman aged 35 years and over and of a man aged 40 years and over (OR = 6.73; 95% CI: 3.50, 12.95) (6). More recently, in a large Danish cohort, a two-fold increase of the risk of early fetal death was found when the father was aged 50 years and over compared with fathers aged 25-29 years, after controlling for various confounders and especially for maternal age (5). In the same cohort, the authors showed a paternal age effect as early as 45 years when considering late fetal deaths.

In a prospective American study of a cohort of more than 5,000 Californian women, the association between paternal age and risk of spontaneous abortion was analyzed by distinguishing between risk of fetal death during the first trimester of pregnancy and risk of fetal death during the beginning of the second trimester (up to 20 weeks of gestation) (20). The authors concluded that elevated paternal age (≥ 35 years) increased the risk of spontaneous abortion during the first trimester and at the beginning of the second trimester, with a suggestion that the association was stronger for deaths occurring during the first trimester.

Interestingly, there is a remarkable concordance in all these studies, stressing the fact that older fathers (≥ 40-45 years) have a key impact on both reproductive issues, failure to conceive and miscarriage. The mechanism for the paternal age effect remains to be explained. Previously, as for maternal age, the genetic

hypothesis had been emphasized (21, 22). After analysis of 11,535 pregnancies obtained by artificial insemination using donor spermatozoa, an increased risk of trisomy 21 for the fetus when the donor was aged ≥38 years has been suggested (23). The aneuploidy rate for both sex chromosomes and for autosomes 9 and 18 was also investigated by comparing 15 men aged 30 years and less and 8 men aged 60 years and older (24), but no significant differences between the two age groups were revealed in this recent study. So, the effect of paternal age on aneuploidy remains debatable and insight into this question may be gained in the future from analysis of aneuploidy mechanisms (25).

Cytogenetic analysis of semen specimens collected from donors has demonstrated an increased risk of frequency of numerical and structural aberrations in men aged 59-74 years compared with men aged 23-39 years (26). More recently, a review indicated that the paternal age effect may be mediated principally by structural chromosomal aberrations in sperm (27). Several authors have also suggested an increased risk of autosomal dominant diseases in children of fathers aged 40 years and older (28). Male genetic alterations could be mediated by age-related increases in germ cell mutations, impairment of DNA repair mechanisms and apoptotic processes (29-32).

On the other hand, morphological changes in the testis have been shown in aging men with decreased numbers of Leydig cells, arteriosclerotic lesions, thickening and hernia-like protrusions of the basal membrane of the seminiferous tubules, and fibrotic thickening of the tunica albuginea (33). These alterations in male reproductive tract function could induce a decrease in quality and quantity of spermatozoa production. A review comparing sperm parameters in men aged under

30 years and over 50 years demonstrated a clear decline in semen volume, sperm motility and sperm morphology with increasing age (34).

Once again, our results confirmed the well-established maternal age effect on the risk of failure to conceive (13). After controlling for paternal age, we found a clearly increased risk of failure to conceive in women after 37 years, in agreement with the literature (12). The cut-off at 37 years was also confirmed by the observed rate of oocyte atresia. Investigation of the number of follicles contained in ovaries obtained during surgery or in women who died suddenly had shown that the disappearance of ovarian follicles accelerated strongly after age of 37.5 years, at the time when the number of follicles fell below the critical figure of 25,000 (14).

The maternal age effect had been principally linked to genetic alteration of oocytes, especially abnormalities in the meiotic spindle of the oocyte, in women aged 37 years and older (15). More recently, the roles of cohesin and the premature separation of homologous chromatids have been put forward. Chromosome segregation during meiosis and mitosis is certainly one of the most important molecular and cellular processes that allow cells to transmit their genetic information across generations. Failure to maintain genetic stability during cell division leads to cell death or malignant transformation. Several authors have demonstrated a role of cohesin (a multi-subunit complex) in sister chromatid cohesion (16-18).

In industrialized countries, a tendency to postpone childbearing has been observed, leading to parents who are more advanced in age. Furthermore, the number of older couples requesting ART procedures has also increased. It had long been known that these couples must be informed that postponing childbearing beyond the age of 35 years for the woman significantly increases the risk of an adverse reproductive outcome (13). It now appears that this is only one aspect of the

age issue. In reproduction, age must no longer be considered as the affair of the woman, but as that of the couple. Just like maternal age over 35 years, paternal age over 40 years is a key risk factor in reproduction.

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Table 1. Adjusted odds ratio (OR) and 95% confidence interval (95% CI) of risk of failure to conceive after IVF attempts in a logistic regression model without maternal/paternal age interaction (*n* = 1,938)

Maternal age (years)			
< 30	(*n*=378)	1.00	
30-34	(*n*=654)	0.99	(0.73 – 1.36)
35-37	(*n*=428)	1.23	(0.85 – 1.77)
38-40	(*n*=302)	1.59	(1.05 – 2.42)
> 40	(*n*=176)	2.21	(1.28 – 3.80)
Paternal age (years)			
< 30	(*n*=276)	1.00	
30-34	(*n*=597)	1.52	(1.08 – 2.14)
35-39	(*n*=585)	1.32	(0.92 – 1.89)
≥ 40	(*n*=480)	1.70	(1.14 – 2.52)

Table 2. Adjusted odds ratio (OR) and 95% confidence interval (95% CI) of risk of failure to conceive after IVF attempts in a logistic regression model with maternal/paternal age interaction (*n* = 1,938)

Paternal age (years)	Maternal age (years)				
	< 30	30-34	35-37	38-40	> 40
< 30	1.00 (reference) (*n*=145)	0.79 (0.42, 1.51) (*n*=63)	1.62 (0.57, 4.57) (*n*=27)	1.29 (0.48, 3.43) (*n*=27)	0.49 (0.16, 1.50) (*n*=14)
30-34	1.44 (0.84, 2.46) (*n*=152)	1.34 (0.84, 2.13) (*n*=283)	1.49 (0.78, 2.85) (*n*=86)	1.47 (0.65, 3.33) (*n*=45)	5.34 (1.22, 23.42) (*n*=31)
35-39	0.78 (0.40, 1.50) (*n*=59)	1.24 (0.76, 2.02) (*n*=205)	1.33 (0.80, 2.22) (*n*=180)	3.05 (1.44, 6.48) (*n*=93)	2.16 (0.89, 5.20) (*n*=48)
≥ 40	1.25 (0.43, 3.62) (*n*=22)	1.36 (0.75, 2.46) (*n*=103)	2.00 (1.10, 3.61) (*n*=135)	2.03 (1.12, 3.68) (*n*=137)	5.74 (2.16, 15.23) (*n*=83)