ANDROLOGY

Delayed Motherhood Increases the Probability of Sons to be Infertile

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Purpose: To ascertain whether advanced maternal age at birth is associated with offspring infertility.

Methods: A written questionnaire was completed by infertile couples attending our clinic in the presence of a researcher.

Results: Maternal age at birth (odds ratio 1.236, 95% CI 1.100–1.388) and birth order of all respondents and their respective siblings (odds ratio 0.551, 95% CI 0.351–0.865) were significant predictors of male infertility. The probability of a man being infertile increased as mother's age at birth increased (regression coefficient $\pm$ standard error 0.212 ± 0.059 ; $P < 0.001$), but decreased as birth order increased (regression coefficient $\pm$ standard error -0.596 ± 0.230 ; $P = 0.010$).

Conclusions: Delayed motherhood may enhance the probability of sons to be infertile. The probability of a man being infertile would be greater if he comes from a small family than if he comes from a large family.

KEY WORDS: male infertility; maternal aging; mitochondria; offspring; oocyte aging.

INTRODUCTION

It is known that in mammals inheritance of mitochondria and mitochondrial DNA (mtDNA) is maternal because sperm mitochondria are ubiquitinated inside the oocyte cytoplasm at fertilization and later subjected to proteolysis during preimplantation development (1). Furthermore, epidemiological evidence shows that recombination with paternal mtDNA is very rare (2). On the other hand, it has been proposed that oocytes may be affected by the systemic (chronological) aging of women (3,4). Such a chronological aging of women may inflict an oxidative damage to oocyte mitochondria and so increase the probability that the resulting offspring carry a subpopulation of defective mitochondria and express mtDNA diseases including congenital sensorial hearing loss, cerebellar ataxia, type I (insulin-dependent) diabetes mellitus, and Alzheimer's disease (5). The present study aims to ascertain whether advanced maternal age at birth is also associated with offspring infertility.

MATERIALS AND METHODS

A written questionnaire was completed in the presence of the researcher (E. Vidal) by infertile couples from the same geographical area (Barcelona, Cataluña) attending our clinic between September 1998 and February 2000. The questionnaire was designed to ascertain the fertility status of his/her respective siblings, cause of infertility, age of parents at

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Table I. Maternal Age at Birth and Birth Order of Respondents and Their Respective Siblings

	<i>n</i>	Maternal age at birth (years)	Birth order	Number of siblings
Men	83	28.5 ± 6.1 ^a	2.4 ± 1.6	3.8 ± 1.4
Fertile	47	26.5 ± 5.3	2.5 ± 1.8	3.8 ± 1.4
Infertile	36	30.4 ± 6.4 ^b	2.3 ± 1.3	3.8 ± 1.4
Oligozoospermia	6	28.7 ± 8.1	1.8 ± 1.0	3.2 ± 0.8
Asthenozoospermia	3	33.0 ± 3.6	3.0 ± 2.7	3.3 ± 1.2
Oligo-asteno-teratozoospermia	16	32.3 ± 6.8 ^c	2.4 ± 1.3	4.9 ± 1.3 ^d
Azoospermia	6	28.2 ± 4.8	2.0 ± 0.9	2.3 ± 0.5
Female factor	5	27.6 ± 5.2	2.2 ± 1.1	3.0 ± 1.0
Women	89	29.8 ± 5.6	2.9 ± 2.3	4.0 ± 2.0
Fertile	59	29.9 ± 5.8	2.8 ± 2.1	4.0 ± 1.8
Infertile	30	29.6 ± 5.2	2.9 ± 2.8	4.1 ± 2.3
Bilateral tubal obstruction	14	30.5 ± 5.9	3.2 ± 3.3	4.5 ± 2.7
Endometriosis	8	27.0 ± 4.7	2.5 ± 1.9	3.0 ± 1.1
Anovulation	2	30.0 ± 1.4	2.5 ± 2.1	2.0 ± 0.0
Male factor	6	30.8 ± 4.2	3.0 ± 3.0	5.5 ± 1.6

^a Values are means ± standard deviations.

^b Value significantly different from fertile men ($P = 0.003$).

^c Value significantly different from fertile men ($P = 0.012$).

^d Value significantly different from azoospermic men ($P = 0.001$).

birth, and birth order of the respondent and his/her respective siblings and parents. A total of 93 out of 111 infertile individuals eligible for the study answered the questionnaire. Individuals were considered infertile if (i) either never had a baby despite having ≥ 1 year of unprotected sexual intercourse or the only children they had were conceived during infertility treatment and (ii) they were the only members of the couple having being diagnosed as infertile according to the work-up for the investigation of the infertile couple outlined by the American Fertility Society (6).

Data from fertile and infertile siblings sharing the same biological mother were collected and entered into the study to control for possible confounders such as maternal reproductive behavior and genetic factors. Infertile subjects were compared with their fertile siblings used as the control group. Brothers and/or sisters who never had sexual intercourse or had protected intercourse, as well as couples with unexplained infertility, were excluded from the study. A total of 83 men and 89 women entered into the study (see Table I for detailed description of causes of infertility, maternal age at birth, birth order, and number of siblings).

Statistical Analysis

Binary logistic regression model with random effects, performed with the Epidemiological, Graphics, Estimation and Testing Package (EGRET, SERC Version 1.02, Seattle, WA), was applied to predict the fertility status of respondents and their respective sib-

lings from a set of explanatory variables including number of siblings, age of parents at birth, and birth order of respondents and their respective siblings and parents. Fertility status, male or female, was coded infertile = 1, fertile = 0; gender male = 1, female = 0; all other variables were entered as continuous variables. All of these variables that were significant in a backward strategy were included in the final models of the sample overall and by gender. Analysis of variance (ANOVA), carried out using the Statistical Package for Social Sciences (SPSS, Inc., Chicago, IL), was applied for comparisons of means. Bonferroni test (when the variances were assumed to be equal) and Dunnett's T3 test (when the variances were assumed to be unequal) were applied to perform post hoc pairwise multiple comparisons between groups. Levene test was used to test the homogeneity of variance. Significance was defined as $P \leq 0.05$.

RESULTS

Table I shows that maternal age at birth of respondents and their respective siblings was significantly higher in infertile men and, in particular, in men suffering from oligo-astheno-teratozoospermia when compared to fertile men. Number of siblings of oligo-astheno-teratozoospermic men was significantly higher than that exhibited by azoospermic men. Random-effects logistic regression analysis evidenced that number of siblings, paternal age at birth, and parents' birth order were not significant predictors of

Table II. Multivariate Logistic Regression Model With Random Effects Predicting Male and/or Female Infertility

Dependent variable	Factor	Regression coefficient (standard error)	P-value	Odds ratio (95% CI)
Male and female infertility ^a	Maternal age at birth	0.094 (0.035)	0.008	1.098 (1.025–1.177)
	Birth order	–0.238 (0.120)	0.047	0.788 (0.623–0.997)
Male infertility ^b	Maternal age at birth	0.212 (0.059)	<0.001	1.236 (1.100–1.388)
	Birth order	–0.596 (0.230)	0.010	0.551 (0.351–0.865)
Female infertility ^c	Maternal age at birth	–0.011 (0.053)	0.838	0.989 (0.891–1.098)
	Birth order	0.012 (0.151)	0.935	1.012 (0.752–1.362)

^a All respondents, male and female, were entered into the analysis.

^b Only the men were entered into the analysis.

^c Only the women were entered into the analysis.

offspring (both male and female) infertility. In contrast, maternal age at birth and birth order of respondents and their respective siblings were significant factors (Table II). The effect of these variables, however, depended on gender of respondents and their respective siblings. In particular when data were sorted out by gender, maternal age at birth and birth order of respondents and their respective siblings were associated with male infertility, but they had no effect on female infertility. It is important to note that the regression coefficient in the model predicting male infertility was positive for maternal age at birth but negative for birth order of respondents and their respective siblings. In other words, the probability of a man being infertile increased as mother's age at birth increased, but decreased as birth order increased.

DISCUSSION

Data from the present study show that advanced maternal age at birth is associated with a higher risk of male offspring to be infertile. This result supports a preliminary study showing that women ≥ 40 years of age have higher odds of conceiving infertile sons because of asthenozoospermia when compared to women under 20 (7). However, we have not observed any association between maternal age at birth and female infertility. This outcome contrasts with other studies reporting that advanced maternal age at conception is linked to daughters displaying menstrual disorders (mean cycle length ≥ 42 days or ≤ 21 days, or a within-subject variation between cycles of ≥ 14 days, or amenorrhea) (8) and, likely, longer interval from wedding to first birth (9). In the present study, the existence of any effect of delayed motherhood on female infertility may have been concealed by the fact that approximately half of the infertile women entered into the study displayed bilateral tubal ob-

struction. It is known that this cause of infertility may be better explained by postnatal factors such as trauma, foreign-body reactions, ischemia, hemorrhage, endometriosis, and most often recurrent or persistent infection by microorganisms such as *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Mycoplasma hominis*, *Ureaplasma urealyticum*, *Mycobacterium tuberculosis*, and endogenous anaerobes (10,11), rather than by preconceptional changes in mother's oocytes.

Although some studies have not found a correlation between woman's age and mtDNA rearrangements in oocytes, others have reported increased rate of mtDNA deletions in ovarian tissue and oocytes from older and/or postmenopausal women when compared to ovaries/oocytes from younger women. Moreover, preovulatory oocytes from middle-aged women show increased mitochondrial numerical density, mitochondrial volume ratio, and mitochondrial profile area, suggesting, therefore, subtle but generalized changes in the oxidative phosphorylation capacity (5). It is important to note at this point that mitochondrial dysfunction may affect normal follicular development and oocyte maturation, and potentially interfere with chromosomal segregation during the first meiotic division (12). Furthermore, irregular mitochondrial function is associated with multiple endocrine abnormalities including hypogonadism (13).

Although it remains to be ascertained whether maternal aging is associated with increased levels of mtDNA mutations in spermatozoa of offspring, it has been found that spermatozoa from asthenozoospermic men display mitochondria with ultrastructural abnormalities (14), substantially lower activities of complexes I, II, and IV of the mitochondrial respiratory chain (15), and lower mitochondrial membrane potential (16) than spermatozoa from control individuals. It also appears that point mutations in the mitochondrial tRNA leucine (UUR) gene and mtDNA rearrangements including deletions and

duplications are associated with oligo-, astheno-, or oligoasthenozoospermia [(17,18); for review, see Ref. 5]. Recently, it has been also shown that the mtDNA haplogroups H and T are significantly more abundant in nonasthenozoospermic and asthenozoospermic populations, respectively (19). Interestingly, activities of complexes I and IV of the mitochondrial respiratory chain, which are partially encoded by mtDNA, are lower in the haplogroup T population compared to the haplogroup H, whereas activity of complex II, which is encoded by nuclear DNA, does not show any difference between groups (19).

We should note that the presence of maternal-age-induced defects in oocyte mtDNA is not the only mechanism capable of explaining offspring infertility. Age-related changes in the maternal reproductive and endocrine system may also cause infertility in offspring. In fact, it has recently reported that grown offspring of early adolescent and middle-aged female mice have lower body weight and delayed puberty, and males have smaller reproductive organs than those produced by young adults (20). In that study (20), it was also observed that female offspring of mothers that become pregnant in either early adolescence or middle age, in turn, produced pups significantly lighter than female offspring of young-adult mothers.

Alternatively, offspring infertility may be induced by an age-associated accumulation of chromosomal aberrations and mutations in nuclear DNA of oocytes. Several experimental and epidemiological studies support this notion. For instance, it has been shown that advanced maternal age (i) is an important factor for instability of nucleotide triplet repeats in mammalian DNA (21) and (ii) is associated with leukemia and brain cancer in offspring (22). However, evidence that supports a role for genetics in human infertility is rare, although three genes have been implicated on genetic infertility in men: the DAZ, RBM, and DFFRY genes (for review, see Ref. 23).

CONCLUSION

Data from the present study show that delayed motherhood may increase the probability of sons to be infertile. This effect may be explained by inheritance of excess loads of maternal defective mitochondria allocated to testis-forming tissues, age-related changes in the maternal reproductive and endocrine system, and/or age-associated accumulation of chromosomal aberrations and mutations in nuclear DNA of oocytes.

Birth order also appears to be a factor associated with male infertility. Our data shows that the probability of a man being infertile would be greater if he comes from a small family than if he comes from a large family, assuming their mothers were the same age when they were born.

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