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Ageing and sperm function

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To evaluate the fertilizing capacity of spermatozoa from elderly men, ejaculates from 29 older fathers (mean age 50.3 years) were compared with those from 35 younger fathers (mean age 32.2 years). In addition to conventional semen parameters, sperm functions were studied that have been reported to be positively correlated with the fertilization rate: progressive motility, acrosin activity, inducible acrosome reaction, and chromosome condensation. Sperm concentration and follicle stimulating hormone concentration differed significantly in both groups. With regard to sperm functions there were no differences between older men and younger men, except for decreased sperm motility in the older group which, however, reached nearly normal values according to World Health Organization criteria. Decreased fertility of older couples is obviously more dependent on the age of the female partner. The significance of genetic risks remains to be clarified, especially when methods of assisted reproduction are applied.

Key words: ageing male/assisted reproduction/genetic risks/male fertility/sperm function

Introduction

There is growing interest in age-related changes of male fertility, because more and more couples wish to have children in their late reproductive years. While female fertility drops significantly after the age of 35 years, male fertility usually persists well into old age, although there are considerable inter-individual variations (Holstein *et al.*, 1988). Morphological changes in ageing testes include fibrosis of the tubular membrane and formation of diverticula; the number of type A spermatogonia decreases, whereas atypical spermatogonia and giant spermatogonia become more frequent. In addition, increased desquamation of immature germ cells and a higher number of malformed spermatids and giant spermatids are observed. Sertoli cells show a higher content of lipids and increased vacuolic degeneration (Holstein *et al.*, 1988). These changes lead to a gradual decline of the daily sperm production and impaired semen quality in elderly men. Most studies on this subject have only reported on changes in the common sperm parameters (Schwartz *et al.*, 1983). However, concerning the increasing rate of assisted reproduction, investigation of sperm function is also required (Aitken *et al.*, 1982). Therefore, the present study was aimed particularly at potential age-related changes of sperm function that are essential for fertilization.

Materials and methods

The study included 29 men aged between 45 and 69 years (mean 50.3) who were already fathers and wished to have further children (mostly because of a new partnership). A group of 35 younger fathers (26–35 years, mean 32.2) served as a control. Patients with varicoceles, antisperm antibodies and inflammatory signs in their ejaculates were not included. In addition to hormonal analyses and conventional semen parameters, which were assessed after sexual abstinence of 3–5 days, the following sperm functions were investigated: (i) progressive motility by light microscopy [grade A, normal value > 25%; World Health Organization (WHO), 1992]; (ii) acrosin activity by gelatinolysis (normal halo diameter >10 μm; Henkel *et al.*, 1995); (iii) inducibility of acrosome reaction by 16 h incubation at a low temperature (4°C) and by the triple stain technique (normal value >7.5%; Talbot and Chacon, 1981; Henkel *et al.*, 1993); (iv) chromatin condensation by Aniline Blue staining (normal value <25% Aniline Blue-positive spermatozoa; Terquem and Dadoune, 1983; Haidl and Schill, 1994).

The better of two initial spermiograms (which were not significantly different) was chosen for the study in order to compare the best possible fertility status. Statistical analysis was carried out by use of the non-parametric Wilcoxon test.

Results

Analysis of conventional sperm parameters showed reduced motility in the older group (23% progressive motility on average) compared to the younger men (30% progressively motile spermatozoa). The sperm concentration was lower in the older (66×10^6/ml) versus the younger men (115×10^6/ml). No differences in sperm morphology were observed between the two groups (Table I). The inducible acrosome reaction and chromatin condensation were not different between older and younger men, although acrosin activity appeared to be higher in the older group; all values were normal (Figures 1–3). Hormone concentrations were also within the normal range; however, the older group showed significantly higher follicle stimulating hormone (FSH) concentrations (Table II). There were no significant differences in the length of time of sexual abstinence (4 versus 4.5 days).

Discussion

It is well known that male fertility gradually declines with advancing age. Recently, the cumulative conception rate after

Table I. Routine spermiogram parameters. Data are means ± SD, with range in parentheses

	Older group (>45 years, n = 29)	Younger group (<35 years, n = 35)	P value
Volume (ml)	3.22 ± 1.7 (0.8–8.5)	3.23 ± 1.5 (1.0–8.0)	0.8185
Sperm concentration ($\times 10^6$/ml)	66.9 ± 66.6 (2.0–293)	115.1 ± 103 (5.6–496)	0.0108
Motility (%) (WHO category A)	23.1 ± 13.7 (2.0–51.0)	30.4 ± 14.5 (0.0–60.0)	0.0417
Morphology (% normal forms)	20.7 ± 16.2 (0.0–62.0)	26.5 ± 13.9 (0.0–60.0)	0.0762

Table II. Hormone analyses. Data are means ± SD (range)

	Older group (>45 years, n = 26)	Younger group (<35 years, n = 33)	P value
Follicle stimulating hormone (mIU/ml)	7.1 ± 3.9 (2.0–16.9)	4.6 ± 2.3 (0.5–9.7)	0.0143
Luteinizing hormone (mIU/ml)	3.3 ± 2.6 (1.2–14.5)	3.0 ± 1.6 (0.5–8.3)	0.7853
Testosterone (ng/ml)	4.6 ± 1.5 (2.7–8.2)	4.9 ± 2.1 (2.0–12.5)	0.5825

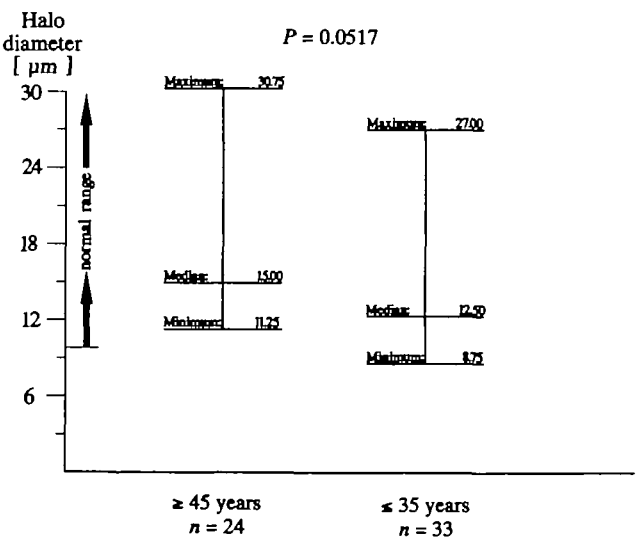

Figure 1. Comparison of acrosin activity in spermatozoa of older (>45 years) and younger (<35 years) men.

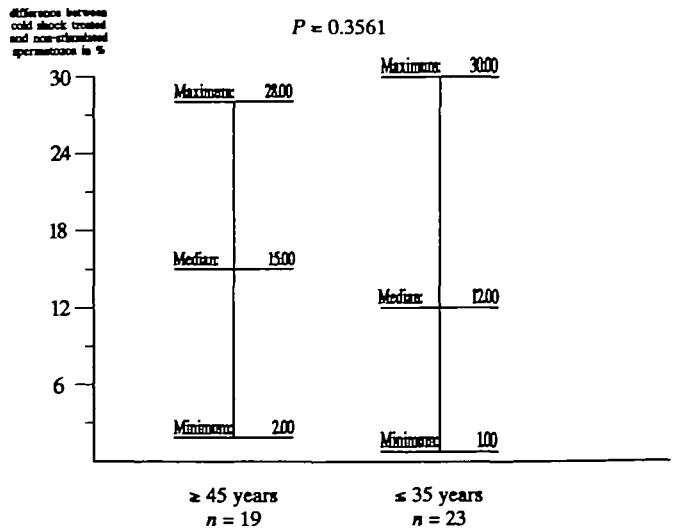

Figure 2. Comparison of inducible acrosome reaction in spermatozoa of older (>45 years) and younger (<35 years) men.

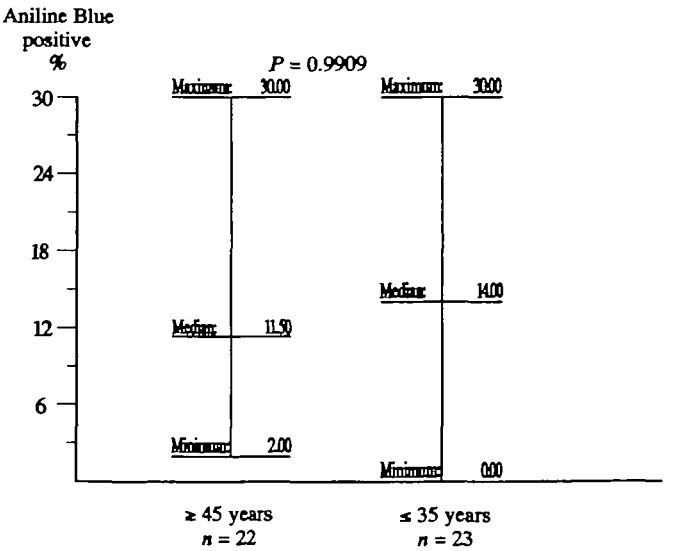

Figure 3. Comparison of chromatin condensation in spermatozoa of older (>45 years) and younger (<35 years) men.

intrauterine inseminations was reported to be lower with paternal age >35 years (Mathieu *et al.*, 1995). A decrease in both sperm motility and percentage of normal-shaped spermatozoa was demonstrated, which, however, had no significant impact on fertility (Nieschlag *et al.*, 1982; Schwartz *et al.*, 1983). Moreover, age-related changes in FSH and luteinizing hormone (LH) concentrations have been documented (Wide, 1985). Some of these findings were confirmed by our study. Sperm motility in the older group was significantly lower than in the younger group, although normal values according to WHO criteria were reached. This observation is in accordance with other studies explaining the reduced sperm motility by a longer time of sexual abstinence in the older men (Nieschlag *et al.*, 1982). In our study, the time of abstinence was not different between the two groups (4 days on average). Additional factors that might influence sperm motility, such as antisperm antibodies, increased viscosity, low sperm volume or infections, were excluded. Thus, no explanation can be given for the lower

motility in the older group. Impaired epididymal function, resulting in a lower number of fully matured and motile spermatozoa, may have contributed to this phenomenon. While sperm concentrations were also significantly lower in the older group, these were within normal ranges. The two groups did not show differences in sperm morphology, which was measured according to the Düsseldorf criteria (Hofmann and Haider, 1985); the normal morphology percentages of 21 and 27 respectively represent 'normal' values (Haidl and Schill, 1993).

To gain more insight into the fertility potential of elderly men, sperm function tests were performed. These are required for an appropriate prognosis of fertility, especially when methods of assisted reproduction are considered. Therefore, our study primarily aimed to use functional tests that have been positively correlated with the success of in-vitro fertilization, e.g. acrosin activity (de Jonge *et al.*, 1993), acrosome reaction (Henkel *et al.*, 1993), and chromatin condensation (Haidl and Schill, 1994).

No differences were observed in acrosome reaction and chromatin condensation; acrosin activity was apparently higher in the older group. All values were within the normal range in both groups. This indicates that the limiting factor for a couple's fertility in the late reproductive years is the age of the female partner (Schill *et al.*, 1994). However, it has to be pointed out that paternal age >45 years has been associated with an increased genetic risk. A high incidence of structural chromosome anomalies in men >44 years was observed, with ~13% of spermatozoa showing chromosomal damage (Martin and Rademaker, 1987). Therefore, the American Association of Tissue Banks established an upper age limit of 40 years for semen donors; the age limit set by the American Fertility Society is 50 years (Bordson and Leonardo, 1991). On the other hand, from studies of live newborns or prenatally diagnosed fetuses, there is no evidence so far that older fathers have an increased frequency of offspring with de-novo (non-inherited) structural chromosomal anomalies (Bordson and Leonardo, 1991).

At any rate, there is one comforting argument in favour of advanced paternal age: the literature and studies of high school boys have indicated that descendants of older fathers, and particularly of older paternal grandfathers, are more intelligent (Dietz-Helmers, 1974).

In view of the new techniques of assisted reproduction, e.g. intracytoplasmic sperm injection, which achieve fertilization even in difficult cases, more data about paternal age and the outcome of such procedures are required. Since the genome of each individual spermatozoon cannot be investigated in advance, an increased risk may exist in old age, and this needs further investigation.

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