Molecular aspects of declining sperm motility in older men

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Objective: To investigate the molecular mechanism for declining sperm motility in older men.
Design: Retrospective and prospective study.
Setting: University-based andrology unit.
Patient(s): Semen was collected from 2,111 patients and sperm donors.
Intervention(s): None.
Main Outcome Measure(s): Age, sperm concentration, ejaculate volume, sperm motility (including velocity average path, velocity straight line, velocity curvilinear, linearity, and lateral head displacement), normal sperm morphology, percentage of abnormally blue-stained flagella, and total and free T, FSH, and LH concentrations were analyzed. In addition, the zinc concentration in the seminal fluid, flagella, and the whole spermatozoa was measured by means of atomic absorption spectrometry.
Result(s): Patient age correlated negatively with T concentration and sperm motility, including velocity, but positively with the percentage of abnormally stained flagella and the flagellar zinc. Whereas the percentage of abnormally stained flagella correlated negatively with motility and sperm velocity, there was a positive relationship with the flagellar zinc content. Flagellar zinc content was negatively correlated with motility. Testosterone showed a positive relationship with motility and sperm concentration.
Conclusion(s): Because the epididymis is functionally T-dependent, our data suggest that the 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. (Fertil Steril® 2005;84:1430–7. ©2005 by American Society for Reproductive Medicine.)

Key Words: Aging men, testosterone, sperm motility, outer dense fibers, zinc

Aging of the female gonads is a well-defined phenomenon, which is closely associated with a decline in reproductive functions, increased rates of miscarriage and chromosomal abnormalities in oocytes, and with decreased embryo implantation rates (1–3). This process begins in the late 20s, becomes more rapid in the late 30s (4), and eventually culminates in menopause, which is characterized by a drastic decline in serum E_2 concentrations (5) at approximately 50 years of age. In contrast, male reproductive functions do not cease abruptly but continue lifelong, and it is not uncommon that men even in their 70s or 80s can father children (for review see Schill [6] and Kühnert and Nieschlag [7]). However, apart from significant morphological changes in aging testes (8, 9), a distinct decrease in the number and function of Leydig cells (10, 11) with a decrease in mitochondrial steroidogenesis (12) and an increase in the serum concentration of sex hormone-binding globulin (13, 14) have been reported. As a result, the T concentrations decrease (15), with an annual rate of approximately 0.8% for total T and 1.7% for biologically active free T (14).

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With regard to the reproductive function and fertility of elderly men, contradictory reports have been published. Whereas some investigators found decreased values for ejaculate volume, sperm concentration, and motility in aging men (16, 17), Krause and Habermann (18) could not find any changes. In contrast, some groups even observed increased sperm concentrations in older men (19, 20). Nevertheless, male fertility is maintained until very late stages in life. Undoubtedly, however, fertility and pregnancy rates decrease even in men, though this is normally attributed to female aging.

Although there have been contradictory reports from oocyte donation models (21), which should eliminate the female age factor, most investigators have found a distinct decline in male fertility (22–24). De la Rochebrochard and Thonneau (25) even speak about paternal age as an important risk factor for infertility if men are aged >40 years. On the other hand, it seems that methods of assisted reproduction that are more invasive, like IVF (21) or intracytoplasmic sperm injection (26), are less associated with paternal age than IUI (27), which requires a much greater amount of highly functional spermatozoa. This raises the question of whether, and more importantly, how and by which molecular mechanism, sperm functions are affected by paternal age. To

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date only few studies, which mainly focused on genetic risks, have addressed this problem.

Apart from sperm morphology, for which a significant age-dependent decrease has repeatedly been reported (for review see Kidd et al. [28]), sperm penetration into zona-free hamster oocytes (19), the acrosome reaction, acrosin activity, and sperm chromatin condensation (29) showed no difference between young and elderly men. On the other hand, Singh et al. (30) demonstrated a significant increase of DNA fragmentation in aged men. For motility, the most obvious function of spermatozoa, a significant decline has repeatedly been described in older men (for review see Kidd et al. [28]), and it is also well known that sperm motility is a good predictor for outcome in assisted reproduction. However, molecular reasons for the decline in sperm motility in older men have not yet been investigated. Therefore, this study aimed at extending the relatively small (200 patients) preliminary comparative study by Jung et al. (31) and investigating a molecular rationale that explains decreased sperm motility in aging men.

MATERIALS AND METHODS

This study consists of two parts. In the first, retrospective part, a total of 2,111 different patients attending the outpatient clinic for fertility problems (aged 17–66 years) were analyzed for sperm concentration (n = 2,106), volume of ejaculate (n = 2,094), total motility (World Health Organization [WHO] A + B + C) (n = 1,695), progressive motility (WHO A + B) (n = 1,695), normal sperm morphology (n = 1,655), abnormally blue-stained flagella (n = 1,649), and concentrations of free T (n = 337), total T (n = 1,355), FSH (n = 1,320), and LH (n = 1,317). Morphological evaluation of the spermatozoa was performed according to the Düsseldorf classification (32, 33) after Shorr stain. Follicle-stimulating hormone, LH, and total T were measured by means of standard ELISAs (Biochem, Freiburg, Germany), whereas the free T was measured by means of RIA.

In the second, prospective part of the study, 157 ejaculates of healthy sperm donors and patients (aged 21–57 years) were collected after 3–5 days of sexual abstinence. After liquefaction for 30 minutes at 37°C, a standard semen analysis, including a morphological evaluation (percentage of morphologically normal sperm and abnormally blue-stained flagella) of the spermatozoa, was performed in all the subjects and a motility analysis by computer-aided sperm analysis (CASA) in 90 ejaculates. Subsequently, the ejaculate was centrifuged for 15 minutes at 300 × *g*. To eliminate remaining particles, seminal plasma was centrifuged again for 20 minutes at 4,000 × *g* at room temperature and eventually frozen at −80°C until further use.

The sperm pellet was resuspended in 500 μL isotonic KCl (Merck, Darmstadt, Germany; suprapure) and centrifuged for 15 minutes at 300 × *g* twice. Afterwards, sperm concentration was adjusted to 25–75 × 10^6/mL, and the flagella were dissolved by addition of an equivalent amount of 0.1 mol/L KOH (Merck, pro Analysi; p.A.) in 90 subjects. Samples were incubated at room temperature for 10–15 minutes and vortexed occasionally. Thereafter, they were centrifuged for 15 minutes at 600 × *g*. The pellet was washed twice with isotonic KCl. The supernatant containing flagella and the pellet containing sperm heads were separately frozen at −80°C until zinc was determined.

Lysis of the flagella was controlled by light microscopy. Because of this procedure, we were able to analyze zinc content in both the sperm heads and the flagella, as well as in the seminal plasma (34). The other 67 sperm samples were not separated into sperm head and flagellum, but zinc content was directly measured. All data obtained were evaluated with regard to dependence on the age of the subjects and connected with data obtained in the retrospective part of the study, especially with motility and the hormonal status of aging men.

Determination of Zinc

Zinc was measured with atomic absorption spectrometry (Perkin-Elmer 300, Überlingen, Germany) with the graphite furnace technique under argon at a wavelength of 213.9 nm. Calibration curves were prepared with zinc standard solution (Merck) for standards. Samples were diluted 1:4 (sperm heads), 1:12 (flagella), and 1:1,000 (seminal plasma) or higher with H_2O (p.A.) if necessary. For matrix correction, concentrated nitric acid (Merck, p.A.) was added to a final concentration of 10%.

Motion Analysis

For motion analysis, a semen aliquot of 100 μL was diluted with synthetic human tubal fluid medium according to Quinn et al. (35), which contained 20 mmol/L *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid and 10 mg/mL human serum albumin. Motility parameters were analyzed in at least 100 spermatozoa with a CASA system (Strömberg-Mika Cell Motion Analysis; Mika Medical Equipment, Bad Feilnbach, Germany) with the following settings: number of frames analyzed, 30; minimal number of frames for classification, 15; range of cell size, 35–350 pixels; maximum velocity for immotility, 5 μm/s; local motility, 15 μm/s; calibration factor, 390. At least 100 spermatozoa were evaluated. The following motility parameters were analyzed: percentage of total and progressive motility, percentage of nonlinear motility, velocity average path (VAP), velocity straight line (VSL), velocity curvilinear (VCL), and amplitude of the lateral head displacement (LHD).

Statistics

After testing for normal distribution of the raw data with the Kolmogorov-Smirnov-test, Spearman rank correlations were determined between the assessed parameters. Results are described as median (means ± SD). To confirm a tendency