A histomorphometric and cytogenetic study of testis from men 29–102 years old

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Objective: To evaluate the influence of aging on testicular histology and the aneuploidy rate in testis postmeiotic cells.
Design: Comparison between older men and younger men.
Setting: Deceased donors and patients who requested assisted reproductive technology (ART).
Patient(s): Thirty-six older men (61–102 years old) and 10 younger men (29–40 years old).
Intervention(s): Testes were obtained from 35 deceased donors, and testicular biopsies were obtained from 11 patients who requested ART.
Main Outcome Measure(s): Histomorphometry of testis and fluorescent in situ hybridization (FISH), with a three-probe set X, Y, and 18.
Result(s): The histomorphometric study showed a thickening of the basal membrane when spermatogenesis was arrested. The number of germinal and Sertoli cells decreased as age increased. The rate of aneuploidy of postmeiotic cells was 1.1% for the control group, 1.29% for older subjects with preserved spermatogenesis, and 14.28% for the subjects with an arrested spermiogenesis. Only this last figure was higher than the control group.
Conclusion(s): The rate of aneuploidy in older subjects (61–95 years old) with preserved spermatogenesis was not statistically different from that found in the control group; it was increased in older subjects with arrested spermatogenesis. (Fertil Steril® 2005;83:923–8. ©2005 by American Society for Reproductive Medicine.)

Key Words: Older men, testis, histomorphometry, postmeiotic cells, FISH, aneuploidy

The majority of men, beginning at puberty, produce sperm with continuous cell divisions throughout their lives. The number of germinal cell divisions therefore increases with age. It is reasonable to hypothesize that the increase in cell divisions due to age increases the mutation frequency, leading to various genetic alterations at the germinal level (1). Women, in contrast, have a discontinuous oocyte production, which involves fewer cell divisions and decreases in their late 30s (2). At around 50 years of age, a woman's fertility stops (i.e., menopause). The number of divisions preceding oocyte production, regardless of a woman's age, is estimated to be 24, whereas in a 45-year-old man, the number of divisions that precede spermatozoa production is about 770 (1). This important difference allows men to have children later in life but most likely with an increased risk of genetic damage. Various studies on the relationship between semen quality and age have demonstrated a decline in semen volume and spermatozoa motility as well as changes in spermatozoa morphology (3, 4). On the other hand, histologic investigations of testes removed from older men reported systemic arteriosclerosis (5, 6) and involution of Leydig cells with advancing age. A positive correlation between the proportion of altered Leydig cells and the decrease in testosterone level was also found (7, 8). Moreover, older men were found to have thicker basal membranes of the seminiferous tubules (9), together with alterations in Sertoli cell structure (10). Finally, the testes of older men showed further degenerative changes of the germ cells and of the germinal epithelium, such as a progressive decrease in spermatozoa numbers or germ cells arrested in the pachytene stage (11–13).

The question of the genetic risk related to paternal age is raised by the increased frequency of medical assisted reproduction techniques (ART) for older men (>50 years old). The deleterious effect of maternal age on abnormal meiotic behaviors is well established (14). In contrast, little is known about the effect of paternal age on chromosomal abnormalities. In fact, results from studies dealing with that question are rather controversial probably because the number of >60-year-old subjects studied were too small to draw solid conclusions. These controversial results have led us to perform the present study, where we have investigated the influence of aging on testicular histology and on the rate of testis meiotic abnormalities evaluated by fluorescent in situ

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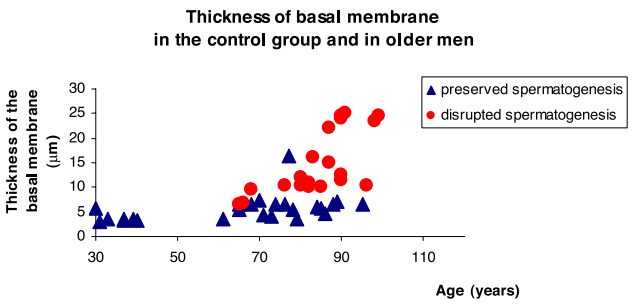
FIGURE 1

Thickness of the basal membrane according to the age and spermatogenesis stage.



Dakouane. Study of testis from men 29–102 years old. Fertil Steril 2005.

hybridization (FISH) for chromosomes X, Y, and 18 in testis postmeiotic cells from older subjects (61–102 years old) and from younger men (29–40 years old). We chose to study chromosomes X and Y for the following reasons: [1] sexual chromosome abnormalities observed in offspring are often from paternal origin (15, 16), [2] XY malsegregations are more frequent in sperm from oligospermic patients than in controls (17, 18), and [3] sexual chromosomes are useful to determine at which step meiosis is arrested (meiosis 1 or 2) or nondisjunction occurs. Cytogenetic analysis of chromosome 18 enabled us to check both the ploidy in the examined cells and the incidence of malsegregation for chromosome 18 where the trisomies are birth living. Moreover, because no comparison has been published so far between cytogenetic and histomorphometric investigations in older testis, the present study also included testes histomorphometric analysis. Finally, we chose to investigate human testes in postmortem because it was the best way to study a large population of older subjects (>65 years old).

MATERIALS AND METHODS

Samples

Testes were removed from 35 deceased donors 65–102 years old, within 24 hours following death. The protocol was included in the Paris Body Donation Program (Department of Anatomy, Centre du Don du Corps des Saint Pères). This study also includes one 61-year-old patient who requested ART after vasectomy.

The control group included 10 younger subjects (29–40 years old) with obstructive azoospermia but showing normal histologic spermatogenesis. All patients consented to this study, which was approved by local institutional Review Board.

Histology

Testes of postmortem donors were cut in longitudinal and transversal sections; two pieces of each testis were fixed in Bouin's solution for histology. After fixation, the pieces of each testis were dehydrated and embedded in paraffin. Histologic sections (7 μm) were performed and then stained by Masson's trichrome.

Morphometric Analysis of Testicular Tissue Components. After a classic study of six histologic sections, a computer-assisted morphometric analysis (Histolab version 5.2.3; Microvision Instrument, Paris, France) was performed based on the following parameters:

[1] For the seminiferous tubules: the surface area, the thickness of the basal membrane, and the nucleus density (Sertoli cells, spermatogonia, spermatocytes, and spermatozoids).
[2] For the interstitial tissue: the cluster numbers, the surface occupied by the Leydig cells, and their nucleus density.

FISH. Slides for FISH were obtained by affixing the testis sections close to Super Frost Plus (CML, Nemours, France) slides (testis print) and then stored at −20°C. A minimum of eight slides were prepared for each testis per subject. For the control group, smears were performed from dilacerated fragments of testis biopsies. Slides were fixed in methanol acetic acid (3/1, v/v) for 5 minutes, and incubated in 2XSSC (300 mM of NaCl, 30 mM of trisodium citrate; pH 7.0) at room temperature for 5–10 minutes. After a 5-minute wash in phosphate-buffered saline (PBS) (131 mM of NaCl, 5 mM of Na_2HPO_4, 1.5 mM of KH_2PO_4; pH 7.2), slides were dehydrated in three graded baths of ethanol (80°, 90°, and 100°) for 1 minute each and then allowed to air dry. Slides were controlled for an adequate number of testis cells by phase contrast microscopy.

No decondensation treatment was applied (19). Commercially available kits including Chromosome Enumeration Probes (CEP) 18 Spectrum Aqua, CEP X Spectrum Green, and CEP Y Spectrum Orange (probes commercialized by Abbott, Rungis, France) were used. The probe mixtures were sealed on the slides, which were then placed in Hybrite 45°C for 2 minutes and then at 73°C for 3 minutes to denature cellular DNA. After an overnight hybridization at 37°C, slides were washed in 0,4XSSC 0,3NP40, pH 7.0 at 73°C for 105 seconds and rinsed in 2XSSC 0,4NP40 for 15 seconds. Nuclei were then counterstained with 4,6 diamino 2-phenylindole (DAPI) from Abbott. The morphologically normal and abnormal cells were then located and ploidy determined according to the number of signals detected for each probe by using a fluorescence microscope (Olympus, Rungis, France) equipped with multiband pass filters. The resulting pictures were acquired through a liquid crystal display (LCD) camera connected to a G4 Macintosh computer system supplied with version 1.4 of the Quips Pathvysion Digital Scientific software.

Statistical Analysis

To analyze the differences in the aneuploidy rates and sex ratio 1/1 between older and younger men, the Mann–Whitney and χ^2 tests were applied using the Stat View program (SAS Institute Inc., Cary, NC).