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# Sperm Structural and Motility Changes During Aging in the Brown Norway Rat

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**ABSTRACT:** The Brown Norway rat provides a useful model to study aging of the male reproductive tract because of the selective age-dependent pathological changes that are found in the testis, epididymis, and prostate. In the testis, there is a clear age-dependent decrease in both steroidogenesis and spermatogenesis. In the epididymis, some striking segment-specific changes occur at the histological and biochemical levels prior to the major loss of spermatogenesis. We hypothesized that formation of spermatozoa in the testis and maturation of spermatozoa in the epididymis (ie, acquisition of motility and loss of the cytoplasmic droplet) may be altered during aging. Changes in the morphology of spermatozoa were assessed by light and electron microscopy. Using computer-assisted sperm analysis, the motility parameters of spermatozoa obtained from the caput and cauda epididymidis of young and old Brown Norway rats were compared. In old animals, we also compared the motility of spermatozoa from epididymides adjacent to regressed testes with those from epididymides adjacent to nonregressed testes. There was a marked increase with age in the number of spermatozoa with abnormal flagellar midpieces; the nature of these defects did not change with age. In caput epididymidis, the percentage of motile sperm was similar in young and old rats. In contrast, the percentage of motile spermatozoa was significantly decreased in cauda epididymidis of old rats; spermatozoa from the regressed testis side had altered motility characteristics. Furthermore, in the cauda epididymidis on the regressed testis side of aged Brown Norway rats, the proportion of spermatozoa that retained their cytoplasmic droplet was markedly elevated. Some of these effects are likely due to changes taking place in spermatozoa during the process of spermatogenesis in the testis (eg, formation of the flagellum), whereas others could occur during sperm maturation in the epididymis (eg, acquisition of motility). The multiple effects of aging on sperm morphology, the acquisition of motility, and the shedding of the cytoplasmic droplet clearly indicate that the quality of spermatozoa is affected by aging.

Keys words: Epididymis, testis, CASA, cytoplasmic droplet, abnormal axoneme.

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The Brown Norway (BN) rat provides an excellent model to study aging of the male reproductive system (Wang et al, 1993; Wright et al, 1993; Chen et al, 1994, 1998; Grunewald et al, 1994; Levy et al, 1999a). Previous studies have reported that BN rats are unusually long-lived, do not develop reproductive tract tumors, and do not become obese as they age; these characteristics make it possible to dissociate age- from health-associated changes in the reproductive tract (Wang et al, 1993; Wright et al, 1993; Grunewald et al, 1994). Aging in BN rats is associated with testicular alterations (Levy et al, 1999a) resulting in a decrease in spermatogenesis and steroidogenesis (Neaves et al, 1987; Zirkin et al, 1993; Chen et al, 1994, 1998). In the epididymis, characteristic signs of aging are observed, such as lipofuscin accumulation and thickening of the basement membrane. In addition, there is a segment- and cell-specific vacuolization and an augmentation in the number and size of lysosomes (Serre and Robaire, 1998a). Some striking changes are observed in the structure and function of the blood-epididymis barrier (Levy et al, 1999b; Serre and Robaire, 1999). Region-specific changes in the expression of genes involved in androgen action, such as steroid 5α-reductases, or enzymes that play a role in detoxification, such as glutathione *S*-transferases, have been reported (Viger and Robaire, 1995, Mueller et al, 1998). Pregnancy outcome studies have revealed that paternal aging causes increases in preimplantation loss, the percentage of low weight fetuses, and postnatal mortality (Serre and Robaire, 1998b). Together, these observations strongly suggest that there are structural changes, functional changes, or both, that occur in spermatozoa as males age.

Mammalian spermatozoa acquire motility and fertilizing capacity during their transit through the epididymis (Orgebin-Crist, 1967; Robaire and Hermo, 1988). Acquisition of motility evolves from a circling motion in the more proximal regions of the epididymis to a linear, progressive motility in more distal regions (Blandeau and

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Rumery, 1964; Hinton et al, 1979). The maximum percentage of motile sperm is attained in the corpus epididymidis in rodents (Yeung et al, 1992), and more distally in the hamster (Kann and Serres, 1980), and the boar (Chevrier and Dacheux, 1992). Sperm maturation is not intrinsic to spermatozoa, but rather requires interaction with luminal epididymal proteins (Dacheux and Volgmayr, 1983; Syntin et al, 1996). In response to changes in the epididymal luminal fluid, an extensive remodeling of the plasma membrane surface of spermatozoa takes place (Jones, 1998). One of the most evident changes is the displacement of the cytoplasmic droplet along the midpiece of the spermatozoon and its subsequent detachment in the corpus and cauda epididymidis (Bloom and Nicander, 1961). There is good evidence to indicate that after release, cytoplasmic droplets are endocytosed by epithelial clear cells (Hermo et al, 1988).

Based on these observations, we hypothesize that structural elements of spermatozoa, their motility characteristics, or both, are altered during aging as a consequence of altered testicular and epididymal age-dependent changes. To test this hypothesis, two approaches were taken. The first was to assess epididymal sperm morphology. Specifically, we assessed the flagellar ultrastructure by electron microscopy and calculated the percentage of sperm retaining their cytoplasmic droplet at the light microscopic level. The second approach was to compare sperm motility parameters of caput and cauda epididymal spermatozoa between young and old BN rats using computer-assisted sperm analysis (CASA).

Our results demonstrate a striking increase in the number of spermatozoa that had abnormal flagellar midpieces in old animals; lesions included alterations in the axonemes, dense fibers, microtubules, and abnormal spatial arrangements of mitochondria. The low motility observed in spermatozoa from caput epididymidis was not altered either quantitatively or qualitatively during aging. The percent of motile spermatozoa from the cauda epididymides of older animals was decreased, and spermatozoa from the regressed testis side had dramatically altered motility characteristics. The proportion of cauda spermatozoa that retained their cytoplasmic droplet was markedly elevated.

## Methods

### Animals

Brown Norway rats aged 3–4 and 20–22 months were purchased from the National Institute on Aging (Bethesda, Md) and supplied by Harlan Sprague-Dawley Inc (Indianapolis, Ind). Rats were housed at the McGill University McIntyre Animal Resources Center under controlled light (14 hours of light, 10 hours of dark) and temperature (22°C). Animals had free access to food and water. All animal studies were conducted in accordance with the principles and procedures outlined in the *Guide to the Care and Use of Experimental Animals*, prepared by the Canadian Council on Animal Care.

In old rats, often one of the testes was smaller than the other and considered as regressed if its weight was less than 1.5 g. The mean testicular weight of nonregressed testes in old rats ($1.95 \pm 0.25$ g) was not significantly different from that of young rats ($1.70 \pm 0.11$ g), but was significantly heavier ($P < .05$) than the weight of regressed testes ($0.98 \pm 0.25$ g).

### Assessment of Sperm Morphology

A comparative study of spermatozoa morphology from adult young and aged BN rats (3, 12, 18, and 24 months) was performed by electron microscopy. Rats were perfused-fixed with 2.5% glutaraldehyde buffered in sodium cacodylate (0.1 M) pH 7.4 in a prograde manner through the abdominal aorta as previously described (Serre and Robaire, 1998a). After perfusion, cauda epididymides were sectioned and cut into 1 $mm^3$ pieces and placed in the same fixative for 2 hours at 4°C. The tissues were washed 3 times in a sodium cacodylate buffer (0.1 M) containing 3% sucrose pH 7.4, postfixed in 1% osmium tetroxide and 1.5% potassium ferrocyanide, and embedded in epoxy resin. Thin sections (70 nm) were cut on an ultramicrotome, counterstained with uranyl acetate and lead citrate, and examined with a Philips 410 electron microscope (Philips, Eindhoven, The Netherlands). Abnormalities in the flagellar midpiece area of spermatozoa present in the cauda epididymal lumen were quantified blindly by examining more than 1000 cross-sections of spermatozoa from tissue blocks made from 3 different animals for each age group.

### Sperm Collection for Motility Analysis

Animals were killed by $CO_2$ asphyxiation. Both epididymides were removed, trimmed free of fat, and placed in a 35 mm Petri dish containing 2 mL of Medium 199 (with Hanks salts; Gibco, Burlington, Ontario, Canada) supplemented with 0.5% bovine serum albumin (crystallized and lyophilized; Sigma-Aldrich Canada, Oakville, Ontario) pH 7.4 at 37°C. Sperm samples were obtained from the distal caput and the distal cauda epididymidis (referred by Miller and Killian, 1987 as region 3b and 6b, respectively) using a ''diffusion'' method (Klinefelter et al, 1991; Slott et al, 1991, 1993). Briefly, each epididymis was clamped with 2 hemostats at the caput-corpus and corpus-cauda junctions, severed proximal to the clamp, blotted, and unclamped. The caput and cauda tissues were transferred to separate Petri dishes containing 3 mL of fresh medium at 37°C. A #11 scalpel blade was used to pierce several tubule segments of the distal caput and distal cauda epididymidis. Due to the fluid pressure within the lumen of the tubule, sperm rapidly moved into the medium. Once turbidity was apparent, at approximately 30 seconds, the tissue was removed and the sperm were allowed to disperse for 5 minutes. The Petri dish was gently swirled and a further dilution (1:10) was made immediately prior to aliquoting into an 80-μm-deep glass cannula for CASA.

### Assessment of Sperm Motility

Sperm were analyzed at 37°C using the Integrated Visual Optical System (IVOS; Hamilton Thorne Research Inc, Beverly, Mass)

equipped with version 10 software. The operational settings were described by Slott et al (1993) and analyses were done for 30 image frames at a rate of 60 frames/sec. Five aliquots were prepared and more than 100 sperm per aliquot were analyzed by IVOS to obtain data on the percentage of motile sperm and primary kinematic parameters, including average-path velocity (VAP), curvilinear velocity (VCL), straight-line velocity (VSL), amplitude of lateral head displacement (ALH), beat cross-frequency (BCF), and 2 derived parameters: linearity (LIN = VSL/VCL $\times$ 100) and straightness (STR = VSL/VAP $\times$ 100).

### *Cytoplasmic Droplet Count*

Using IVOS, sperm were visualized under phase contrast microscopy and monitored on the computer screen; this allowed for clear visualization of cytoplasmic droplets. After spermatozoa stopped moving, screen captures of several fields were made and stored. The cytoplasmic droplets were counted on more than 500 spermatozoa ($n \geq 5$ rats/age group).

### *Statistical Analysis*

Data were analyzed using the Sigmastat 2.3 (SPSS Inc, Chicago, Ill) software package. Unpaired Student's *t* tests were employed to assess whether age caused significant effects. Bonfferoni's correction was applied when multiple *t* tests were done. The level of significance was taken as $P < .05$.

## *Results*

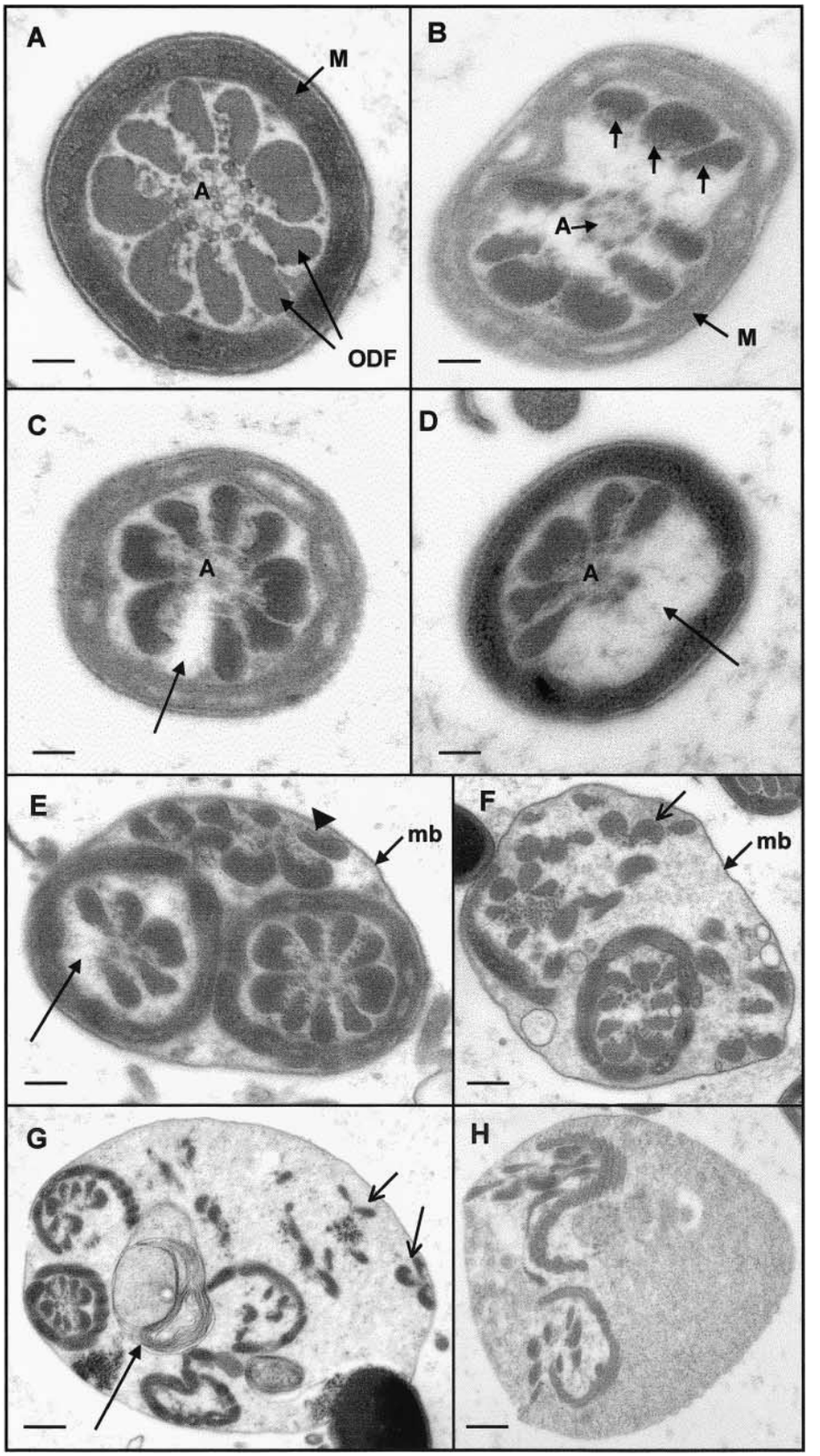
### *Abnormal Axonemal Structures*

The axoneme of a normal sperm is composed of 2 central and 9 peripheral microtubule doublets, and 9 outer dense fibers (Figure 1A). We observed different types of ultrastructural abnormalities in both young and old rats, none of which seemed to be exclusive to old rats. One of the aberrations was the abnormal spatial arrangement of several of the dense fibers to the centrally located axoneme at the flagellar midpiece level (Figure 1B). Modifications in the number of axonemal components and dense fibers were found. In some sperm sections, one outer dense fiber and microtubule doublet were missing (Figure 1C). We also observed the hemilateral absence of dense fibers and some of the microtubules (Figure 1D). In some transverse sections, 2 or more flagellar axonemes enclosed by the same sperm membrane were seen (Figure 1E through G). This alteration in the flagellum was often associated with other abnormalities, such as disorganization of the mitochondrial sheath, axonemes, and dense fibers, or the hemilateral absence of dense fibers and microtubules. Some spermatozoa seemed to be in the process of degenerating (Figure 1H). All types of abnormalities were counted together and a percentage was calculated. The proportion of spermatozoa showing an altered flagellar midpiece or the presence of several tails enclosed by the same membrane gradually increased with age to become significantly higher at 12 months ($P < .05$), and reached a maximal twofold increase by 24 months of age (Figure 2). At the age at which some animals had 1 regressed testis (18 months), no difference was noted either with respect to the types of abnormalities found or the proportion of abnormal spermatozoa.

### *Acquisition of Sperm Motility*

In the young adult (3–4 month) BN rat, we observed that the percentage of motile spermatozoa increased from 16.9% $\pm$ 1.8% (mean $\pm$ SEM) in the distal caput epididymidis to a maximum of 76.8% $\pm$ 1.3% in the distal cauda epididymidis (Figure 3). In addition, the swim paths of spermatozoa changed as they passed through the epididymis; motile sperm from the caput epididymidis showed a circling motion whereas spermatozoa from the distal cauda epididymidis followed a relatively straight path (Figure 4A and B). Kinematic parameters revealed that VSL (which is the distance between the first and the last track points; Figure 4) and STR increased significantly between caput and cauda epididymal sperm (Figure 5A and B). However, for those spermatozoa that were motile, the total distance covered by caput epididymal sperm was similar to that covered by sperm from the cauda epididymidis (Figure 4), as revealed by VCL and VAP (shown for VCL in Figure 5C). In addition, parameters reflecting beat characteristics, such as ALH and BCF, were similar between caput and cauda epididymal sperm (shown for ALH in Figure 5D).

The motility characteristics of caput and cauda epididymal sperm were compared between young rats and old rats from 2 different groups. In the first group of old rats neither testis was regressed (ON-2), whereas the second group had 1 nonregressed testis and 1 regressed testis; spermatozoa from the caput and cauda epididymidis were taken from both the nonregressed testis side (ON-1) and the regressed testis side (OR-1). In caput epididymidis, the percentage of motile sperm was similar between young and old rats with either 1 or 2 nonregressed testes, and on both the nonregressed and the regressed testis sides (Figure 3). The circling motion of these caput epididymal sperm was very similar between young and old BN rats (Figure 4A and C). No significant differences were observed for the various motility parameters analyzed, except for lateral head displacement (Figure 5). In contrast, the motility of spermatozoa from cauda epididymidis of young rats was significantly higher than that from old animals with either 1 or 2 nonregressed testes (Figure 3). The percentage of motile spermatozoa was decreased by approximately 15% in both groups, but the kinematic parameters of motile sperm were unchanged, indicating that fewer spermatozoa were motile, but they reached normal motility characteristics (Figure 5). In contrast, spermatozoa from the cauda epididymidis adjacent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to regressed testes showed an even greater decrease in sperm motility, down to 25% (Figure 3). Their swim paths were affected as shown in Figure 4D, and appeared to be intermediate between caput and cauda epididymal sperm tracks due to a reduction of straightness, velocity, and abnormal lateral head displacement. This was reflected by a significant decrease in all three velocity parameters (straight-line, curvilinear and average path) (Figure 5A and C for the first 2 parameters cited; changes in VAP were similar to that of VCL), as well as STR and ALH (Figure 5B and D). Progressive motility, defined as those spermatozoa having STR greater than 50% and VAP greater than or equal to the medium value velocity (MVV) set at 50 μm/s, was very much lower than percent motility, but provided a similar profile for the various groups. For spermatozoa from the caput epididymidis, values ranged from 1.6% to 2.3% and did not differ from each other. For spermatozoa from the cauda epididymidis, young animals had 26% progressively motile sperm, whereas for old animals, values for ON-2, ON-1, and OR-1 groups were 19%, 19%, and 6%, respectively. It is interesting that none of the motion parameters of cauda epididymal spermatozoa from the regressed testis side were significantly different from those of caput epididymal spermatozoa.

### *Cytoplasmic Droplet Loss*

The cytoplasmic droplets were clearly detectable when spermatozoa were visualized under phase contrast microscopy with the CASA system (Figure 6). In young adult rats, the percentage of spermatozoa having a cytoplasmic droplet declined from 93% in the distal caput to less than 20% in the distal cauda epididymidis (Figure 7). In the caput epididymidis of old animals, whether neither or 1 testis was regressed, 80% of sperm showed the presence of a cytoplasmic droplet on their flagellum; this was significantly lower than the percentage found for young rats. In the cauda epididymidis of old rats adjacent to nonregressed testes, only 25% to 27% of sperm had an attached cytoplasmic droplet; this was similar to that of young rats. In contrast, 40% of spermatozoa from the regressed testis side retained their cytoplasmic droplet, twice the percentage seen in young rats (Figure 6B and D; Figure 7). Thus, whereas the ratio of spermatozoa with a cytoplasmic droplet in the caput epididymidis to that in the cauda epididymidis is greater than 4.5 in young rats, it is only 3 in old rats on the nonregressed side and 2 on the regressed side.

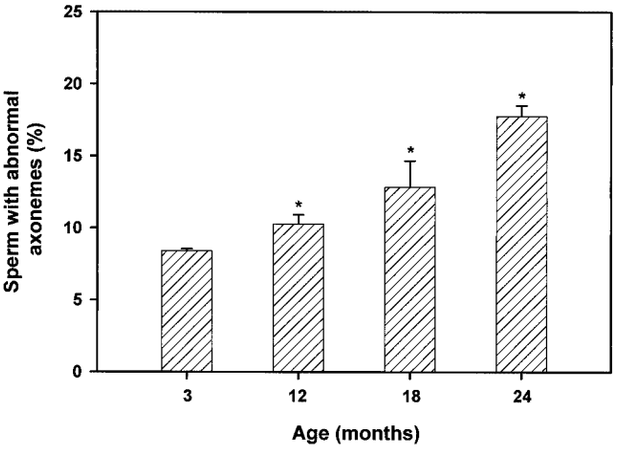


Figure 2. Percentage of cauda epididymal spermatozoa showing an abnormal flagellar midpiece or the presence of several tails surrounded by the same membrane in rats aged 3, 12, 18, and 24 months (n = 3). Data are expressed as mean ± SEM. *Values differ significantly from 3-month-old BN rat by Student's *t* test, $P < .05$.

## *Discussion*

There is a growing interest in the age-related changes that occur in the male reproductive system (Haidl et al, 1996). Although the aging process has been studied extensively for testicular steroidogenesis and prostatic function, little information is available on how aging affects the quality of spermatozoa, either with respect to their formation in the testis or their maturation in the epididymis. We present here, for the first time, an objective analysis of the sperm morphology and motility changes that take place during aging in BN rats.

The ultrastructural abnormalities observed were quite similar to those in adult human (Zamboni et al, 1987), boar (Bonet et al, 1992), and hamster (Calvo et al, 1999). Lesions included abnormal or absent mitochondria and

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Figure 1. Cross-section of the midpiece of spermatozoon flagellum within the lumen of the cauda epididymidis. **(A)** Note the normal spatial arrangement of the 9 outer dense fibers (ODF) to one another, to the centrally located axoneme (A) composed of the 9 plus 2 arrangement of microtubules, and the mitochondrial sheath (M). **(B)** Abnormal spatial arrangement of several outer dense fibers (arrows) to the centrally located axoneme. **(C)** One outer dense fiber and microtubule is missing. **(D)** Hemilateral absence of outer dense fibers and microtubules. **(E)** Three flagella are visible, enclosed by the same membrane (mb). One flagellum appears normal, another has a hemilateral absence of outer dense fibers and microtubules (arrow), the third is completely disorganized with no mitochondrial sheath observable (arrowhead). **(F)** Two flagella are visible enclosed by the same membrane (mb); both show a disorganized mitochondrial sheath, one is opened, releasing the outer dense fibers (arrow with open head). **(G)** Several flagella sectioned through the cytoplasmic droplet in which free mitochondria and outer dense fibers are visible (arrow with open head); there are also axonemal and dense fiber defects as well as the development of lamellar membranes (arrow). **(H)** Two flagella that appear to be undergoing a process of degeneration. Bar = 31 μm (A, C); 37 μm (B, D); 52 μm (E); 79 μm (F); 130 μm (G, H).

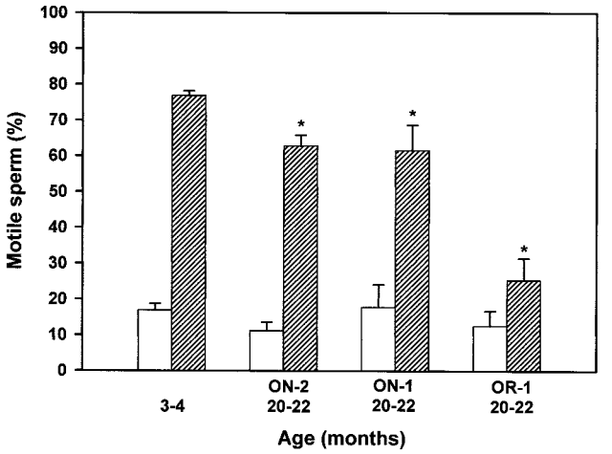


Figure 3. Effect of age on the percentage of motile spermatozoa obtained from the distal caput (light gray bar) and the distal cauda epididymidis (left-hatched bar) of young (3–4 months) and old (20–22 months) BN rats with either 2 (ON-2) or 1 nonregressed testis (ON-1) and 1 regressed testis (OR-1). Percentage of motile sperm is defined as those having VAP greater than 20 μm/s. Data are expressed as mean ± SEM (n = 9, 7, 5, and 5, respectively). *Values differ significantly from young BN rat by Student's *t* test, $P < .05$.

alterations in the axoneme, outer dense fibers, and microtubules. These abnormalities probably reflect an impairment of spermatogenesis and not epididymal maturation, thus spermatozoa entering the caput epididymidis would show the same abnormalities as those in the cauda epididymidis. This was shown to be the case by Trasler et al (1988) for caput and cauda epididymal spermatozoa in the control group of their study; therefore, only cauda epididymal spermatozoa were analyzed to assess whether aging affects sperm ultrastructure. Our quantitative results indicate that the number of defects increases progressively with age. However, no new types of alterations were detected during aging; a similar observation was made for aged hamsters (Calvo et al, 1995). The actual percentage of abnormal spermatozoa is likely to be higher than that reported in Figure 2 because only abnormalities at the midpiece level of the flagellum were taken into account. The increase in the percentage of abnormal flagellar midpieces found in spermatozoa from the epididymis during aging may be due to either induction of the defect in the testis or a decreased ability to reabsorb abnormal sperm in the epididymis.

In young adult BN rats, we observed a characteristic motion for spermatozoa from caput (circling) and cauda epididymidis (progressive) similar to that reported for other strains (Fray et al, 1972; Burgos and Tovar, 1974). Kinematic parameters of BN cauda epididymal sperm appeared to be similar to those shown for Sprague-Dawley rats using similar settings (temperature, media, chamber depth, tracking rate, and duration; Slott et al, 1991; Chapin et al, 1992). As a consequence of aging, there were no significant differences in the already low motility parameters observed for spermatozoa found in the caput epididymidis. In contrast, in the cauda epididymidis, sperm motility declined by approximately 15% on the nonregressed testis side. This is in agreement with some tests performed in human ejaculated sperm (Nieschlag et al, 1982; Haidl et al, 1996; Rolf et al, 1996) and hamster epididymal sperm (Calvo et al, 1999) showing a decrease of sperm motility in the same order of magnitude. On the regressed testis side, the motility was decreased remarkably from 76% in young adults to 25% in old rats. Moreover, the motility characteristics remained similar to those of caput epididymal spermatozoa, suggesting that the acquisition of sperm motility was incomplete in the old BN rat epididymides. Several possible explanations that could account for this observation are that the transit time through the epididymis is decreased, that epididymal functions (eg, absorption, secretion) are defective, thus altering the epididymal microenvironment bathing sperm and therefore impairing the acquisition of sperm motility, or that there are some alterations in spermatogenesis resulting in sperm that cannot acquire normal motility.

Several different factors involved in the development of motility during epididymal transit have been described, such as intracellular pH (Hamamah and Gatti, 1998); intracellular $Ca^{2+}$ (Vijayaraghavan and Hoskins, 1990); cyclic adenosine monophosphate (cAMP; Tash, 1990), and potentially, disulfide bond content (Yeung et al, 1994). The mechanism for the regulation of motility by pH is uncertain, although environmental factors such as the ion composition of the luminal fluid appear critical (Gatti et al, 1993). It has been postulated that the high level of cytosolic $Ca^{2+}$ is responsible for the lack of mobility of immature sperm. Efflux of $Ca^{2+}$ may be affected by plasma membrane channels during epididymal transit. The stimulatory effect of cAMP on sperm motility and its increase in sperm during epididymal maturation have been well established (Cornwall et al, 1986). cAMP has been shown to act via the regulation of protein phosphorylation, including that of axonemal proteins (Tash, 1990). Finally, oxidation of sulfhydryl groups to form disulfide bonds was described as a potential factor involved in the acquisition of sperm motility because ''stiffening'' of the tail of spermatozoa from the caput epididymidis by oxidation of free sulfhydryl groups resulted in an increase in VSL (Yeung et al, 1994). It is likely that at least 1 of these processes is altered in the epididymis adjacent to a regressed testis, leading to incomplete sperm maturation.

Previous studies have reported that expression of several epididymal biochemical markers is impaired in aging BN rats (Viger and Robaire, 1995; Mueller et al, 1998). It is not known how an alteration in sperm movement would affect fertilizing ability (Olds-Clarke, 1996). However, previous studies reported a correlation between

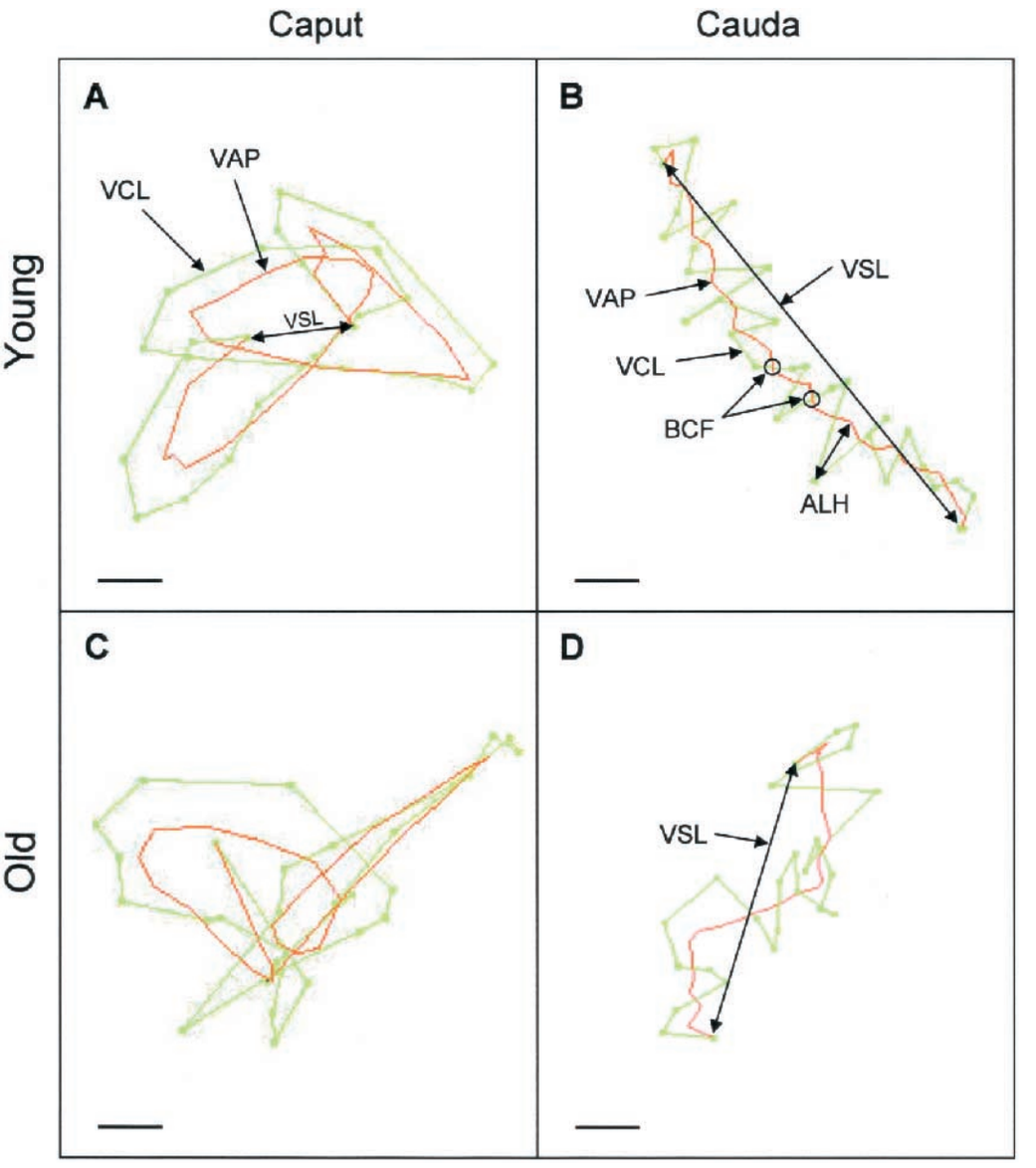


Figure 4. Representative schematic tracks of one spermatozoon obtained from the distal caput **(A, C)** and distal cauda epididymidis **(B, D)** of young animals **(A, B)** and old animals **(C, D)** on the regressed testis side. Analysis was performed for 30 image frames at a rate of 60 frames/s. Bar = 16 μm.

sperm motility parameters and fertility in rats treated with epichlorohydrin (Toth et al, 1991). Such an altered sperm maturation in aged rats might contribute to the defects in progeny outcome observed in the old BN rat (Serre and Robaire, 1998b).

Another end point of epididymal sperm maturation that was assessed is the proportion of spermatozoa that retained their cytoplasmic droplet as they moved from the caput to the cauda epididymidis. Shedding of the cytoplasmic droplet is part of epididymal sperm maturation (Fawcett and Phillips 1969). However, an alteration in this process could be linked to a defect in spermatogenesis, leading to impaired sperm membrane modification. Sperm entering the caput epididymidis are characterized by the presence of the cytoplasmic droplet, which then rapidly migrates along the midpiece of the tail and eventually detaches from the spermatozoon in the corpus and cauda epididymidis (Hermo et al, 1988). Our observations indicate that this process is impaired in old BN rats. In the cauda epididymidis on the regressed testis side, twice as many spermatozoa still had their cytoplasmic droplet attached to their flagellum. The influence of these changes on the acquisition of sperm motility and fertilizing ability remains unclear. However, it has been suggested that a

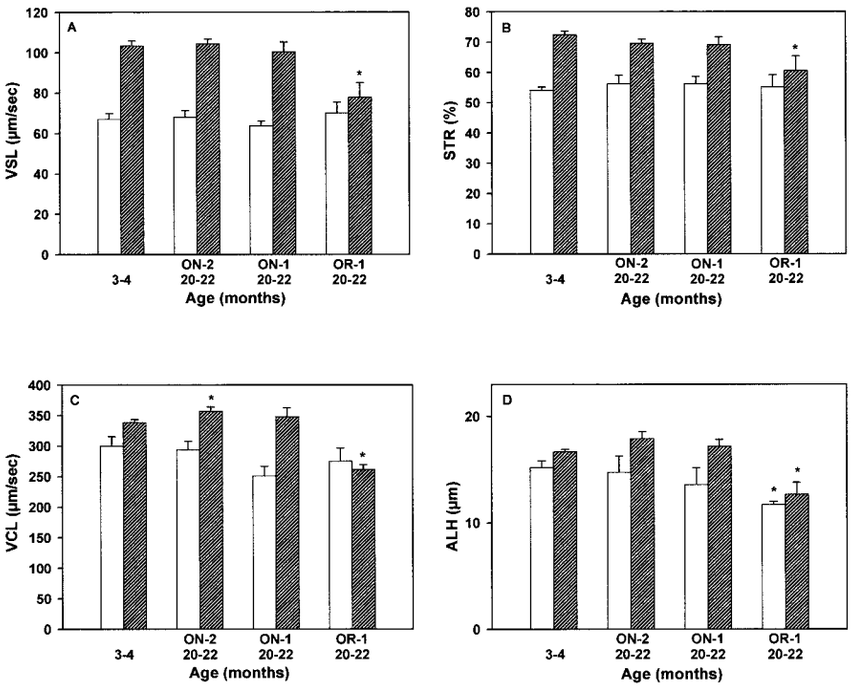


Figure 5. Effect of age on the motility characteristics (ordinates) of spermatozoa obtained from the distal caput (gray bar) and the distal cauda epididymidis (left-hatched bar) of young and old BN rats. Data are expressed as mean ± SEM (n = 9, 7, 5, and 5, respectively). *Values differ significantly from young BN rat by Student's *t* test, $P < .05$.

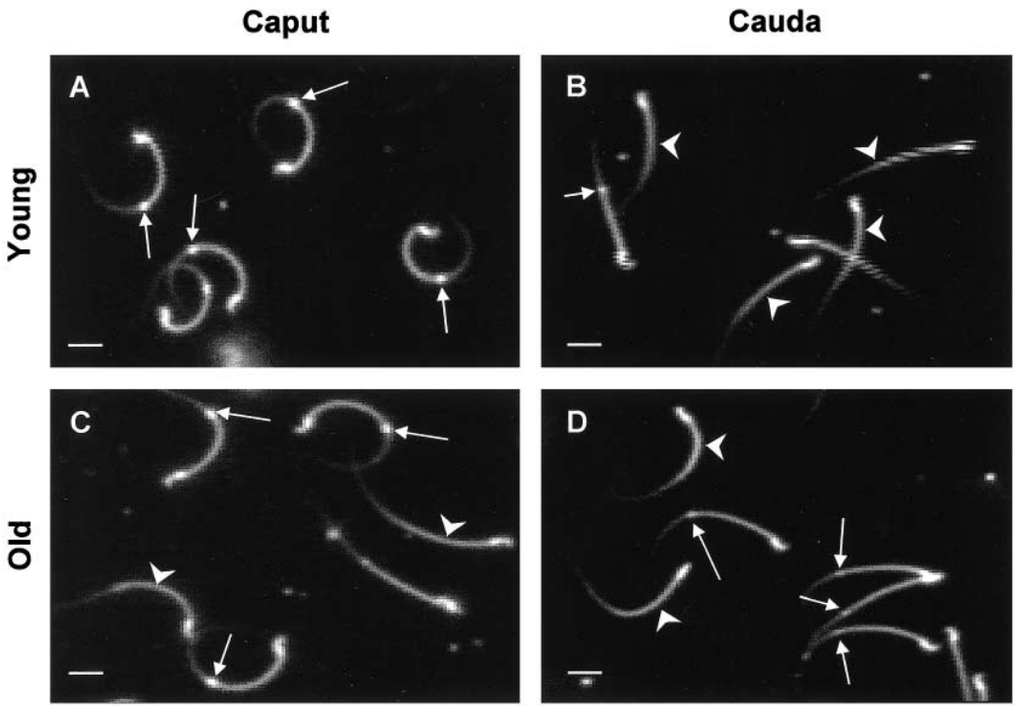


Figure 6. Phase contrast pictures of spermatozoa from the caput (A, C) and the cauda epididymidis **(B, D)** of young **(A, B)** and old **(C, D)** BN rats. Several cytoplasmic droplets are shown with the white arrows, whereas some of the spermatozoa without their cytoplasmic droplet are pointed out with an arrowhead. Bar = 16 μm.

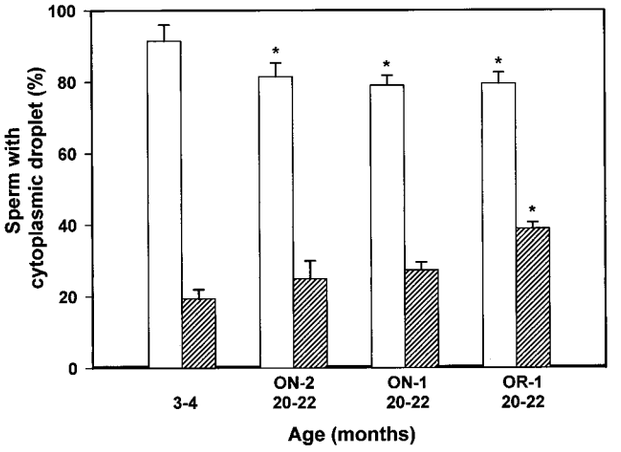


Figure 7. Effect of age on the percentage of spermatozoa that retain a cytoplasmic droplet attached to their flagellum in the distal caput (gray bar) and distal cauda epididymidis (left-hatched bar). The cytoplasmic droplets were counted from light microscopic pictures of more than 500 spermatozoa in at least 5 rats. Data are expressed as mean ± SEM. *Values differ significantly from young BN rat by Student's *t* test, $P < .05$.

high number of spermatozoa with attached droplets in the ejaculate can be correlated with altered epididymal functions and reduced fertility (Dadoune, 1988).

These results clearly demonstrate that there are numerous clear changes that occur in spermatozoa during aging. Furthermore they support the hypothesis that modifications in male germ cells during aging are due to altered functions at both the levels of the testis and the epididymis.

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