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Testicular Steroidogenesis in the Aging Brown Norway Rat

BARRY R. ZIRKIN,* ROSEMARY SANTULLI,* JOHN D. STRANDBERG,†
WILLIAM W. WRIGHT,* AND LARRY L. EWING*‡

*From the *Division of Reproductive Biology, Department of Population Dynamics, The Johns Hopkins University, School of Hygiene and Public Health, and †Division of Comparative Medicine, The Johns Hopkins University School of Medicine, Baltimore, Maryland.*

ABSTRACT: In seeking an animal model of age-associated changes in the male reproductive tract, we examined the effects of age on the health and testicular steroidogenic activity of the Brown Norway rat, with comparisons made to the Sprague-Dawley rat. When perfused *in vitro* under conditions of maximally stimulating luteinizing hormone significant age-associated reductions were seen in testosterone production by testes of Sprague-Dawley rats of 21–24 months of age and by testes of Brown Norway rats of 18–30 months of age. Decreases in the capacity of the testes to produce testosterone were reflected in age-associated decreases in both serum testosterone and in testosterone concentration within the seminiferous tubule fluid. In contrast to the Sprague-Dawley rat, changes in steroidogenic activity in the Brown Norway rat were not accompanied by the occurrence of pituitary adenomas, obesity, or testicular tumors. This, along with its longevity, make the Brown Norway strain a highly promising model for testicular aging.

Key words: Aging, testis, steroidogenesis, Leydig cell, rat.
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Striking changes in steroidogenesis occur with aging of a number of mammalian species, including the human and rat. In the human, reduced serum levels of testosterone, diminished sperm production, and degenerative changes in the seminiferous tubules accompany aging (Ewing, 1975; Harman and Talbert, 1985; Neaves et al, 1987; Tsitouras, 1987; Paniagua et al, 1991). Previous reports have shown that age-associated changes in the male reproductive tract of the rat are similar in important respects to changes in the human; Leydig cell steroidogenic activity and sperm production are reported to decline in aged rats, and degenerative changes in the seminiferous epithelium occur (Harman and Talbert, 1985; Taylor et al, 1986). Unfortunately, however, testicular tumors, pituitary tumors, and obesity are among the age-associated characteristics of many rat strains (Hollander, 1976). Such characteristics make it difficult to distinguish between deficits of the male reproductive tract that result from age and those that result from disease.

We undertook the studies of the Sprague-Dawley and Brown Norway rat strains presented herein to seek an appropriate animal model with which to study testicular aging apart from disease. We show that aging in both strains is accompanied by reduced steroidogenesis, as in the human. In contrast to the Sprague-Dawley and many other previously studied rat strains, Brown Norway rats do not develop reproductive tract tumors, gain little weight, and are long lived, making it feasible to distinguish between age-associated deficits of the reproductive tract and deficits that result from tumors of the hypothalamo–pituitary–gonadal axis that are common to many rat strains.

Correspondence to: Dr. Barry R. Zirkin, Division of Reproductive Biology, Department of Population Dynamics, The Johns Hopkins University, School of Hygiene and Public Health, Baltimore, Maryland 21205.

‡This manuscript is dedicated to the memory of our dear friend and colleague, Larry L. Ewing.

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Materials and Methods

Animals

Sprague-Dawley rats of 6–24 months of age were purchased from Zivic-Miller (Zivic-Miller Corporation, Zelienople, PA). Brown Norway rats of 6–30 months of age were obtained through the National Institute on Aging, supplied by Charles River. Rats were housed in barrier facilities by the suppliers and subsequently at The Johns Hopkins School of Hygiene and Public Health, under controlled light (14 hours light: 10 hours dark) and temperature (22°C) and with free access to rat chow and water.

Animal Pathology

Postmortem specimens were obtained from Sprague-Dawley rats of 6–24 months of age and from Brown Norway rats of 6–30 months of age. These specimens were examined grossly and then were used for histologic examination of digestive organs, upper and lower respiratory tract, salivary glands, endocrine system, and reproductive tract.

Blood Serum and Seminiferous Tubule Fluid

Rats were euthanized by decapitation. Trunk blood was collected, and serum was prepared and stored at −20°C for sub-

sequent determination of testosterone and corticosterone concentrations by radioimmunoassay (RIA). Seminiferous tubule fluid was collected from testes according to the method of Turner et al (1984). In brief, the tunica albuginea was incised at one pole, and testes were centrifuged at low speed (54 × *g*, 10 minutes, 0°C) to drain interstitial fluid. Subsequently, testes were decapsulated and rinsed thoroughly to remove residual interstitial fluid, and the seminiferous tubules were then extruded through the hub of a syringe. This preparation was centrifuged (6,000 × *g*, 15 minutes, 0°C) to collect seminiferous tubule fluid as a supernatant above the tubules. Seminiferous tubule fluid was frozen in liquid nitrogen and subsequently stored at −70°C before assay for testosterone by RIA.

Testosterone—Serum and seminiferous tubule fluid testosterone concentrations were determined in duplicate samples by previously described RIA procedures (Cochran et al, 1981; Turner et al, 1984). The sensitivity of the assay was 10 pg/tube, with intraassay and interassay coefficients of variation of 11.2% and 9.6%, respectively.

Corticosterone—Serum corticosterone concentration was measured by a double antibody RIA, using reagents obtained from ICN-Flow Biomedicals, Inc. (Irvine, CA). All samples and standards (10 μl in 1:200 dilution with assay buffer) were assayed in duplicate. Intraassay and interassay coefficients of variation were 4.4% and 6.5%, respectively.

In Vitro Perfusion

One testis from each rat (N = 10 per age group) was perfused *in vitro*, as described previously (VanDemark and Ewing, 1963; Ewing et al, 1975). The perfusion medium consisted of Krebs–Ringer bicarbonate buffer (pH 7.4) containing 3% (w/v) bovine serum albumin (fraction V, Armour, Inc., Chicago, IL) and washed bovine red blood cells (hematocrit 24). All testes were perfused at 34 ± 0.5°C in a controlled temperature chamber, at a perfusion medium flow rate of 10 ml/g testis/hour. Glucose and luteinizing hormone (NIH-LH-S_{19}: ovine) were infused into the arterial cannula via a Sage micropump (Sage Instruments, Cambridge, MA), so that their final concentrations in the perfusion media were 1.0 mg/ml and 100 ng/ml, respectively. The LH concentration (100 ng/ml) represented the maximally stimulating dose (Zirkin et al, 1980). Testosterone in the testicular venous effluent was measured with a high pressure liquid chromatograph equipped with a flow-through UV spectrophotometer (Cochran et al, 1979).

Statistical Analysis

One-way analysis of variance was employed to detect significant age effects. Scheffé's multiple range test was used to identify differences between groups (Scheffé, 1959). Values were considered significant at $P < 0.05$.

Results

Sprague-Dawley rats in our colony live as long as approximately 27 months. Of the 66 Sprague-Dawley rats evaluated in this study, 27 were 24 months of age when euthanized. Of these, 25% were diagnosed with pituitary adenomas, all were grossly obese (about 1 kg, see below), and some had testicular tumors. The life expectancy of Brown Norway rats in our colony is greater than 36 months. Analysis of postmortem specimens obtained from Brown Norway rats of ages 6–30 months (N = 47) revealed that none of the rats developed testicular or pituitary tumors even through 30 months (N = 9), and none became obese (see below). Moreover, serum concentrations of corticosterone did not change significantly in Brown Norway rats of 6–24 months of age (29 ± 3 ng/ml at 6 months; 44 ± 10 ng/ml at 24 months), indicating that aged rats of this strain did not develop abnormalities of adrenal function that would affect the reproductive tract. In contrast to published reports of other rat strains (Hollander, 1976), bladder neoplasms were not encountered in Brown Norway rats. Indeed, Brown Norway rats remained free of significant nonreproductive tract (adrenal kidney, pancreas, spleen, heart) pathology through 24–30 months of age. By age 30 months, some rats had developed myocardial fibrosis and valvular nodularity, and the most severely affected rats had chronic pulmonary congestion at this time.

Figure 1A,B compares the body weights of aging Sprague-Dawley and Brown Norway rats, respectively. The weight of 3-month-old Sprague-Dawley rats (not shown) was approximately 300 g. By 6 months (Fig. 1A), average body weight rose to almost 700 g (660 ± 26), from age 6 to 12 months weight rose significantly to almost 1,100 g (1,063 ± 43), and by 24 months weight was approximately 800 g (858 ± 54), still significantly greater than at 3–6 months. In striking contrast, the body weight of Brown Norway rats (Fig. 1B) changed relatively little from age 6 to 30 months of age; the average body weight at 6 months was 327 ± 6 g, at 24 months it was 383 ± 10 g, and at 30 months it was 399 ± 13 g. The weights at ages 24 and 30 months were significantly greater than at age 6 months.

Figure 2A shows that the ability of testes of Sprague-Dawley rats to produce testosterone when perfused *in vitro* with maximally stimulating LH, which was 16.3 ± 1.7 nmol/testis/hour at age 6 months, rose, but not significantly, between ages 6 and 18 months, and then was reduced through ages 21 (13.2 ± 1.6) and 24 (10.5 ± 1.4) months. The reduction from age 18 to 24 months was significant. In Brown Norway rats (Fig. 2B), more striking changes in testosterone production by maximally stimulated *in vitro*-perfused testes were seen; testosterone production was 23.3 ± 2.3 nmol/testis/hour at age 6 months, remained unchanged at 12 months (19.8 ± 3.0), was reduced significantly by 18 months (10.1 ± 2.2), and subsequently further decreased, though not significantly, from 18 to 30 months (6.9 ± 1.6 at 24 months; 3.7 ± 0.8 at 30 months).

Serum testosterone levels in Sprague-Dawley rats (Fig.

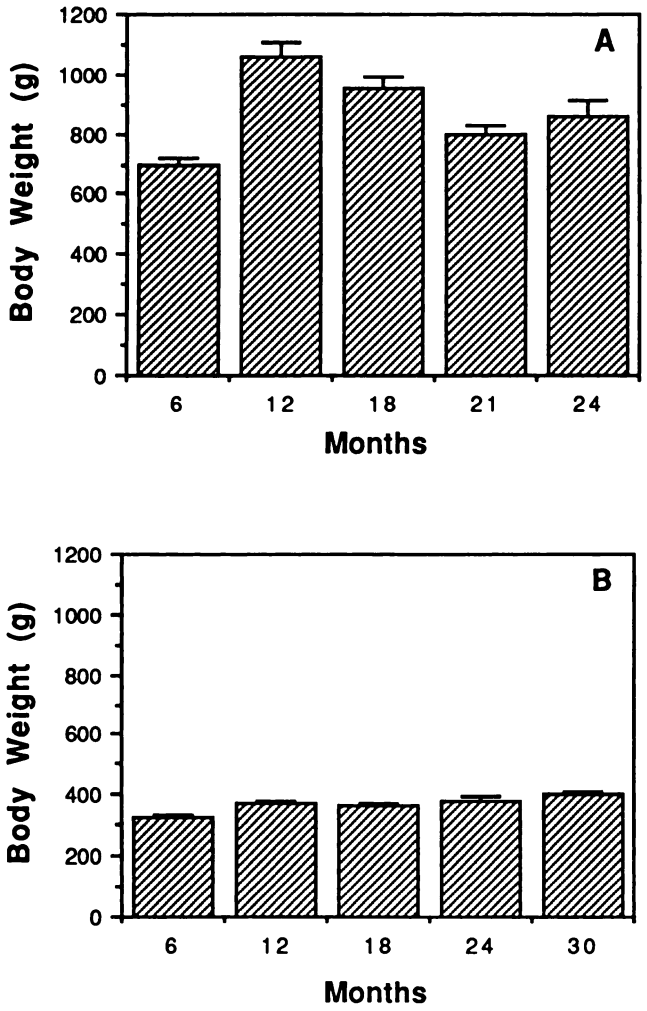

FIG. 1. Body weights of aging rats. **(A)** Sprague-Dawley rats of ages 6–24 months. Mean ± SEM. Weights rose significantly from age 6 to 12 months and remained significantly elevated. **(B)** Brown Norway rats of ages 6–30 months. Mean ± SEM. The weights at ages 24 and 30 months were significantly greater than at age 6 months.

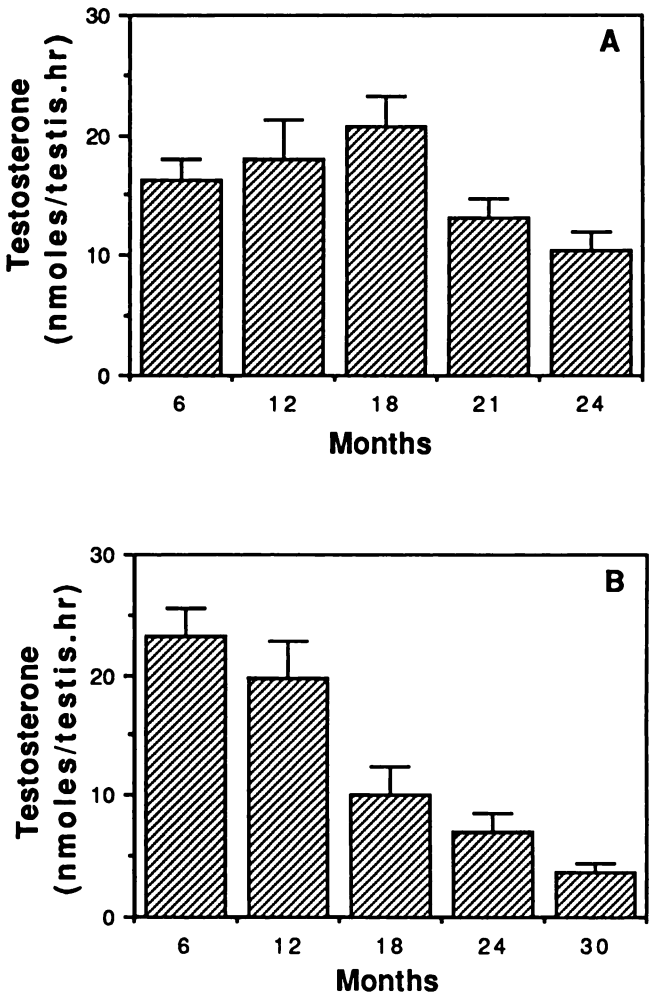

FIG. 2. Testosterone secretion rates by testes of aging rats perfused *in vitro* with maximally stimulating LH. **(A)** Sprague-Dawley rats of 6–24 months. Mean ± SEM. The reduction in testosterone secretion rate from age 18 to 24 months was significant. **(B)** Brown Norway rats of 6–30 months. Mean ± SEM. The reductions in testosterone secretion rates from ages 6 and 12 months to ages 18 through 30 months were significant.

3A) were reduced significantly from age 6 months (2.0 ± 0.3 ng/ml) to age 24 months (0.8 ± 0.1 ng/ml), reflecting the age-associated decreases seen in the capacity of the testes to produce testosterone (Fig. 2A). Serum testosterone levels in Brown Norway rats (Fig. 3B) also were reduced significantly from age 6 months (1.2 ± 0.2 ng/ml) to age 30 months (0.4 ± 0.1 ng/ml), consistent with the decreased capacity of testes from aged rats of this strain to produce testosterone when stimulated maximally with LH (Fig. 2B).

Seminiferous tubule fluid testosterone concentration in the Sprague-Dawley rat (Fig. 4A) was reduced by about 40% from age 6 months (55.0 ± 3.2 ng/ml) to age 24 months (33.2 ± 7.1 ng/ml). These changes, though substantial and reflective of the reduced capacity of the testes to produce testosterone (Fig. 2A), did not reach significance. Similar changes were seen in the seminiferous tubule fluid testosterone concentration of the Brown Norway rat (Fig. 4B), with significant reductions of about 50% from age 6 months (55.4 ± 8.5 ng/ml) to age 30 months (28.4 ± 4.7 ng/ml).

Discussion

Leydig cell steroidogenic activity in the Brown Norway rat, assessed by determining the capacity of the testes to produce testosterone when perfused *in vitro* with maximally stimulating LH, as well as by measuring serum and seminiferous tubule fluid testosterone concentration, diminishes with age. Diminished steroidogenic activity, though less striking, also was seen in the Sprague-Dawley rat (this paper), and occurs in other rat strains (Harman

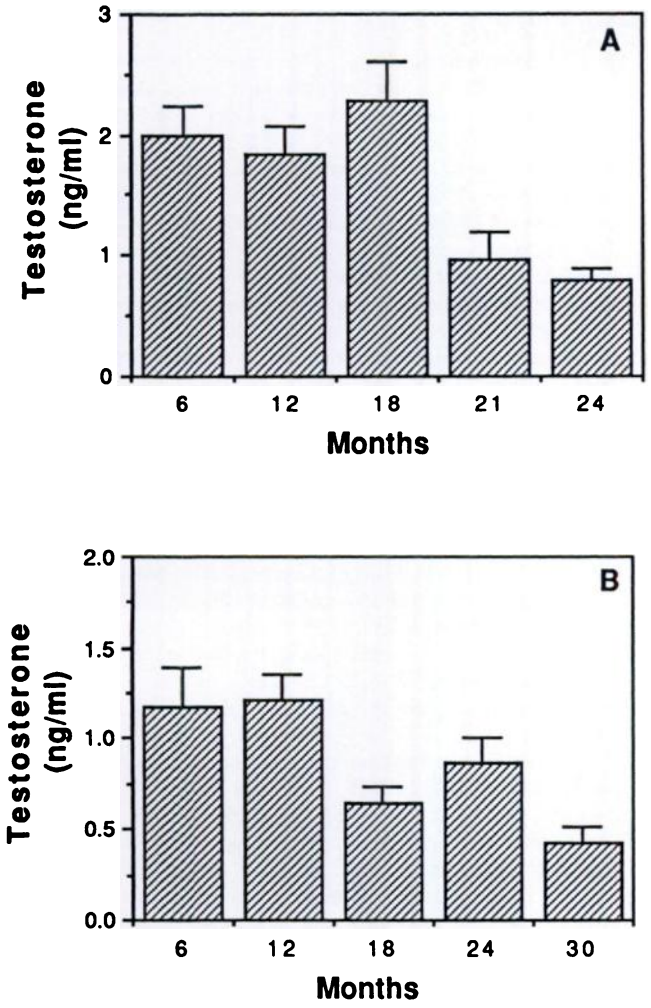

FIG. 3. Serum testosterone levels in aging rats. **(A)** Sprague-Dawley rats of ages 6–24 months. Mean ± SEM. Testosterone levels were reduced significantly between ages 6 and 24 months. **(B)** Brown Norway rats of ages 6–30 months. Mean ± SEM. Testosterone levels were reduced significantly between ages 6 and 30 months.

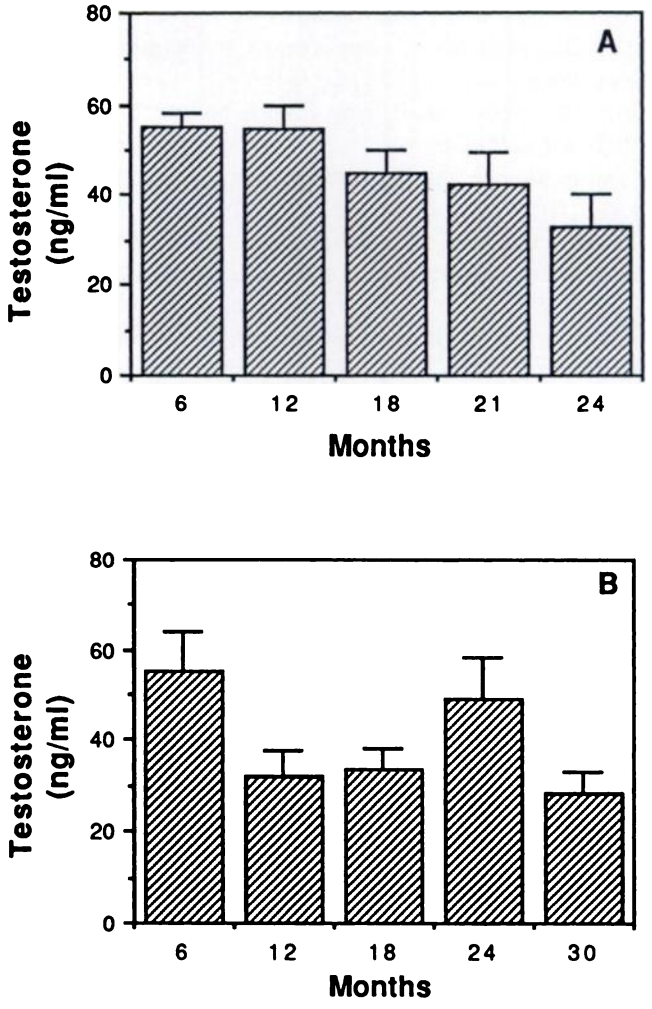

FIG. 4. Seminiferous tubule fluid testosterone concentration in aging rats. **(A)** Sprague-Dawley rats of ages 6–24 months. Mean ± SEM. Testosterone concentration was reduced by 40% (though not significantly) between ages 6 and 24 months. **(B)** Brown Norway rats of ages 6–30 months. Mean ± SEM. Testosterone concentrations were reduced significantly by 50% between ages 6 and 30 months.

and Talbert, 1985). Diminished steroidogenesis also has been reported for the human (Ewing, 1975; Harman and Talbert, 1985). Additionally, degenerative changes in the seminiferous tubules accompanying aging in both the rat (Taylor et al, 1986; Wright et al, 1993) and the human (Neaves et al, 1987; Tsitouras, 1987; Paniagua et al, 1991). Thus, age-associated changes in the male reproductive tract of the rat are similar in important respects to changes in the human.

Unfortunately, however, deficits of the reproductive tract of the rat that may occur as a consequence of aging can easily be confounded by pathophysiologic changes such as tumors of the hypothalamo–pituitary–gonadal axis and obesity. The latter are among the common diseases of the Sprague-Dawley rat and of many other previously described strains of rats. The difficulty in dissociating age-associated from health-associated changes in these strains seriously detracts from their use as models of reproductive tract aging.

In contrast, we have found that Brown Norway rats do not develop reproductive tract tumors and gain little weight through 30 months of age. Increases in lesions of the heart did occur with aging, but such lesions are also commonly seen in aging rats of other strains, and were seen only when the Brown Norway rats were at relatively advanced ages (24–30 months). Importantly, none of the health-related changes that were encountered with aging in males of the Brown Norway strain would be expected to have an impact on the reproductive tract.

The deficits in the male reproductive tract seen in both Sprague-Dawley and Brown Norway rats occurred between ages 18 and 24 months. Brown Norway rats live for more than a year after significant reproductive tract

changes occur and remain healthy through all of this period. This provides the opportunity to examine the etiology of age-associated deficits in reproductive tract structure and function apart from disease and well before the rats become fragile.

In the human, serum gonadotropin levels are reported to rise with aging (Johnson, 1986; Neaves et al, 1987; Tenover et al, 1987; Paniagua et al, 1991), suggesting that the primary age-associated defects in the human reproductive tract occur at the level of the tract itself rather than secondary to involution of the hypothalamus or pituitary. In contrast, studies of aging rats of several strains (Wistar, Long-Evans, and Fisher) have shown that plasma gonadotropin concentrations decrease with age (Harman et al, 1978; Kaler and Neaves, 1981; Sonntag et al, 1984; Harman and Talbert, 1985; Kinoshita et al, 1985). This, along with evidence that Leydig cell deficits may be reversible with exogenously administered gonadotropin (Harman et al, 1978; Vermeulen et al, 1983; Tsitouras et al, 1984), suggest that age-associated deficits in Leydig cell steroidogenic function in previously studied rat strains may result from chronic understimulation by gonadotropin. Therefore, in contrast to the human, the primary testicular age-associated defects in these rats may be at the level of the gonadotropins and not at the level of the testis itself.

In preliminary studies, we found that serum levels of follicle-stimulating hormone (FSH), measured by RIA, rose significantly in the Brown Norway but not in the Sprague-Dawley rat (unpublished data). This observation is consistent with the possibility that Brown Norway rats do not become gonadotropin deficient, and therefore that the observed diminished ability of Leydig cells of this strain to produce testosterone may be intrinsic to the Leydig cell itself. Studies are underway at present to determine whether bioassayable FSH and LH indeed rise with aging of the Brown Norway rat. Whether or not this is the case, however, the similarity of age-associated changes in the male reproductive tract of the Brown Norway rat to those in the human, the longevity of rats of this strain, the absence of pituitary adenomas or testicular tumors, and the absence of obesity and of other commonly observed pathological changes that would make it difficult to distinguish between age-associated deficits of the male reproductive tract and deficits that result from disease make the Brown Norway rat a highly promising model for male reproductive tract aging.

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