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Influence of age on the production of rat spermatozoa, on their concentration in the cauda epididymidis, and on FSH, LH and testosterone plasma levels

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Summary. Testis samples were taken from young (3 months), middle-aged (12 months) and aged (24 months) male rats, processed, stained and examined via a light microscope. There were no prominent anormal germinal epithelium and interstitial tissue. However, the aging process promoted a significant decrease in the mean amount of spermatids 19 per cross tubular section, and in the amount of Sertoli cells per cross tubular section in 24-month-old rats. The concentration of spermatozoa in the cauda epididymidis showed a gradual decrease from 3 to 12 and 24 months. After hCG injection all groups of animals exhibited an increase in plasma testosterone level, although the response was smaller in 12- and 24-month animals compared to the young mature (3 months) ones.

Key words: Testis, Rat, Aging

Introduction

Aging in male rats is accompanied by testicular degeneration, decreased quality of the ejaculated, and infertility. Aging effects in the testis included thickening of the basement membrane of the seminiferous tubules, progressive intratubular fibrosis, thinning or desquamation of the spermatogenic epithelium, and tubular obliteration (see review in Bishop, 1970).

The male rat is characterized by sharply decreased serum testosterone concentration with increased age (Miller and Riegle, 1978; Pirke et al., 1978; Steger et al., 1979). In addition, the aged male rat has decreased serum luteinizing hormone (LH) concentration (Shaar et al., 1975; Riegle and Meites, 1976), decreased hipothalamic-pituitary responsiveness to sistemic L-dopa injection (Watkins et al., 1975a; Riegle and Meites, 1976), and decreased pituitary responsiveness to a single LHRH injection (Watkins et al., 1975b; Riegle and Meites, 1976).

The present study was undertaken to study the production of spermatozoa, their concentration in the cauda epididymidis of aging rats, and FSH, LH and testosterone plasma levels.

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Materials and methods

Young adult (3 months, n = 10), middle-aged (12 months, n = 10), and aged (24 months, n = 10) male Wistar rats were used in this study. The rats had free access to balanced ration and tap water. All animals maintained stable body weight and were free of obvious disease or tumors.

For histological study, 5μm thick sections of testis were stained with ferric hematoxylin. The production of spermatozoa was estimated by the mean amount of spermatids 19 (identified according to Leblond and Clermont, 1952) present in 10 seminiferous tubule cross sections at stage VII, per animal. The amount of Sertoli cells was also recorded, but only when the nucleolus was visible in the section.

In order to estimate the concentration of spermatozoa stored in the cauda epididymidis, the adherent fat, blood vessels and connective tissue were cut away and the organ was placed in a hollow plate. The sperm was released by cutting the cauda epididymidis longitudinally with a pair of fine-pointed scissors and compressing with forceps. The sperm suspension was drawn into a white blood cell pipette and diluted 1:100 with 0.05% collagenase in Ringer-phosphate solution, for 30 minutes, at 34-37°C. After this procedure a final 1:1000 dilution was performed with formol saline fixative (1.8% Nacl and 2.0% formalin). A hemocytometer with improved double Neubauer ruling was then used for the counting of spermatozoa. Counts for 2 to 4 hemocytometer chambers were averaged.

For hormone evaluations, five animals from each group were injected intravenously with saline containing

5 IU hCG (Pregnil, Organon) per 100g body weight, and the five remaining animals received intravenous injections of saline, and were used as controls. Blood samples (2 ml), taken under light ether anesthesia, were collected from the jugular vein, between 9:00 and 11:00 am, immediately before (basal level) and 60 minutes after hCG or saline treatment. Plasma was obtained by centrifugation and stored at -20°C until assayed for LH, FSH and Testosterone (T).

Plasma T level was measured in blood samples taken before and 60 min after hCG or saline injections, by the double-antibody radioimmunoassay method of Bēlanger et al. (1980 a,b) slightly modified, after extraction with purified ethyl ether. FSH and LH were also measured by double-antibody radioimmunoassay (Niswender et al., 1968), only in blood samples taken before hCG or saline injections.

Testosterone 7 α Buitirate-TME and NIAMD-rat FSH-I-5 and NIAMD-rat-1H-I-6 were iodinated by the chloramine-T method (Greenwood and Hunter, 1963). The first testosterone antibody and Testosterone-TME were gifts from Dr. A. Bēlanger (CHUL, Quebec, Canada), and the second antibody was obtained from Dr. J. Antunes-Rodrigues (Faculty of Medicine of Ribeirão Preto-USP, Ribeirão Preto, Brasil). The material used for FSH and LH determinations was supplied by Dr. A.F. Parlow through the NIAMDDK rat pituitary program. All samples were measured in duplicate in the same assay. The intra-assay error was 7.2%, 6.4% and 5% for LH, FSH and testosterone respectively. FSH and LH concentrations were expressed in terms of NIAMD-FSH-rat-RP_2 and NIAMD-LH-rat-RP_2 standards.

Statistical analysis

Comparison of the results were made by the non-parametric Mann-Whitney test (Siegel, 1975).

Results

No signs of lesion were detected upon microscopic observation of testis from aging rats. Histological features from young mature (Fig. 1A), middle-aged (Fig. 1B) and aged (Fig. 1C) rats revealed qualitatively normal germinal epithelium and interstitial tissue.

However, by means of quantitative evaluation it was possible to detect that the aging process promoted a significant decrease in the mean amount of spermatids 19 per cross tubular section at 24 months, when compared with groups of 3 and 12 months (Fig. 2). The mean amount of Sertoli cells per cross tubular section also presented a significant decrease at 24 months of age (Fig. 2). The concentration of spermatozoa in the cauda epididymidis showed a gradual decrease from 3 to 12 and 24 months, attaining an intermediary state at 12 and a nadir at 24 months of age (Fig. 3).

Plasma LH and T levels exhibited significantly lower values at 12 and 24 months of age. Plasma FSH presented a gradual decrease from 3 to 12 and 24 months, exhibiting an intermediary value at 12 and a minimum at 24 months of age (Fig. 4).

FIGURE 1

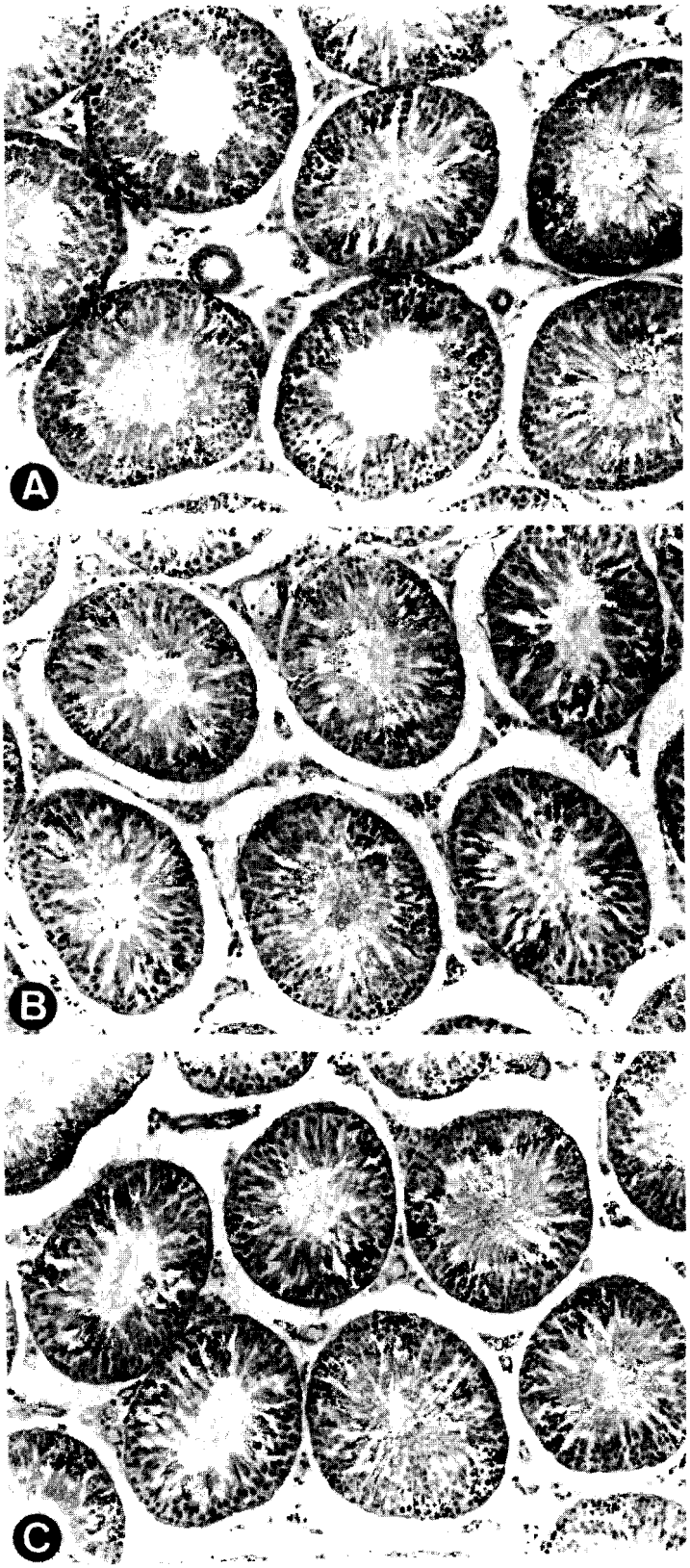

Fig. 1. Histological features of rat testis at 3 (A), 12 (B) and 24 (C) months of age. Ferric hematoxylin. × 250

FIGURE 2

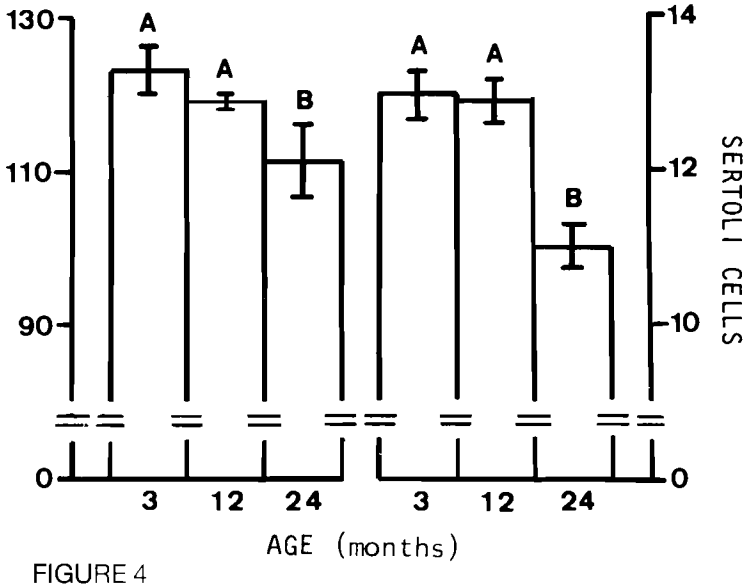

FIGURE 3

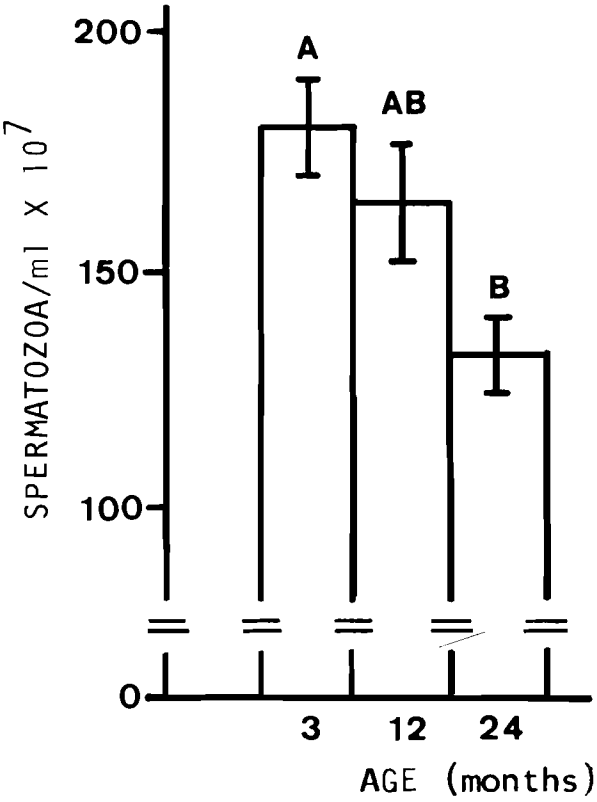

FIGURE 4

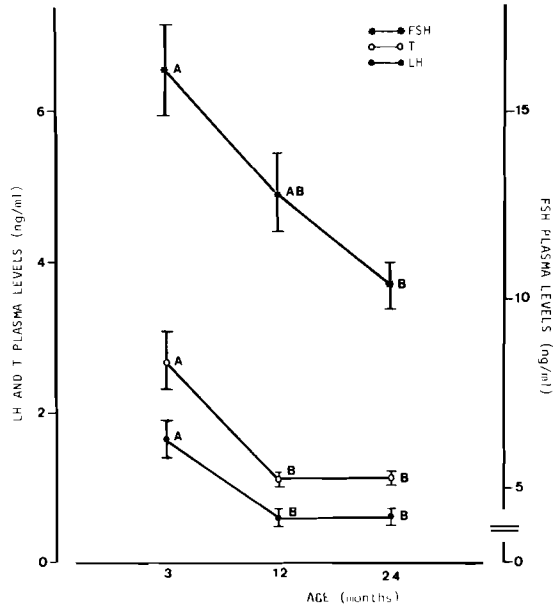

FIGURE 5

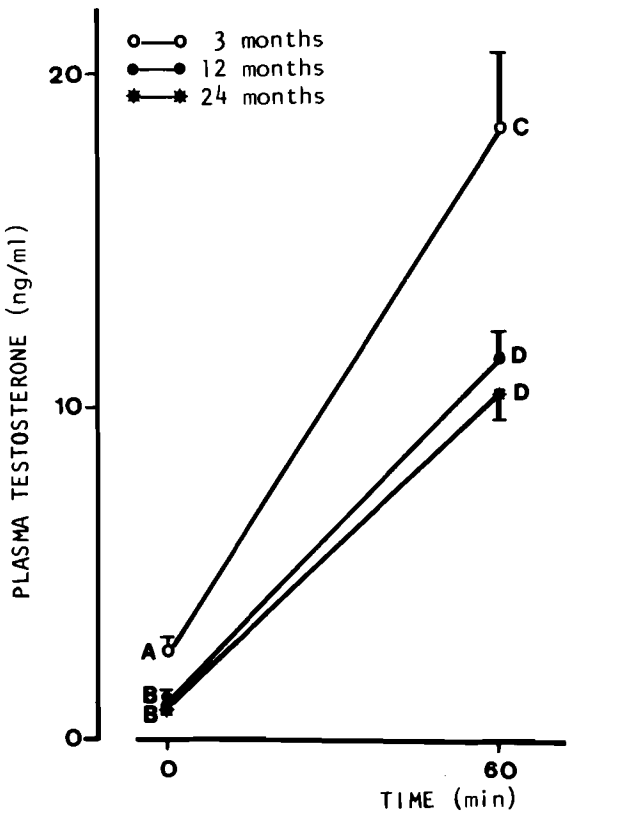

Fig. 2. Amount of spermatids 19 and Sertoli cells per seminiferous tubule cross section, at stage VII of the germinal epithelium cycle, of rats at 3, 12 and 24 months of age (n=5 in each group). Mean ± SEM. For each parameter, A is different from B ($p < 0.05$).

Fig. 3. Concentration of spermatozoa in the sperm stored in the cauda epididymidis of rats at 3, 12 and 24 months of age (n=5 in each group). Mean ± SEM. A is different from B, and AB does not differ from A or B ($\alpha = 0.05$).

Fig. 4. FSH, LH and testosterone (T) plasma levels in rats at 3, 12 and 24 months of age. Each point represents the mean ± SEM value of 10 animals. For each hormone measurement A is different from B ($p < 0.05$) and AB does not differ from A or B.

Fig. 5. Effect of hCG stimulation (5 UI/100g, i.v.) on plasma testosterone levels in rats at 3, 12 and 24 months of age. Each point represents the mean ± SEM value of 5 animals. Blood samples were taken before (time zero) and 60 minutes after hCG injection. $A \neq B \neq C \neq D$ ($P < 0.05$)

The testicular response to hCG stimulation is plotted in Fig. 5. After 60 min of hCG injection all groups of animals exhibited an increase in plasma T level, although the response was less in the 12-and 24-months-old animals as compared to the young mature (3 months) group. Testosterone levels remained stable in control groups of all ages that received saline injections (not shown in the figure).

Discussion

The present data revealed that aged (24 months) male Wistar rats have decreased amount of spermatids 19 and Sertoli cells per seminiferous tubules cross section (stage VII). The seminiferous tubules are lined with spermatogonia which undergo mitotic division to produce primary spermatocytes. These latter cells divide meiotically producing secondary spermatocytes and spermatids which, without further division, mature into spermatozoa. Each of these cell types is arranged in well-defined cellular associations within the seminiferous epithelium, thus composing «stages of the seminiferous epithelium». These stages succeed one another in a cyclic fashion not only the length of the seminiferous tubules, but also in a given area of the epithelium. Each complete sequence of changes in cellular associations is called «cycle of the seminiferous epithelium». In rats the cycle consists of 14 stages, and in stages VII and VIII there are step 19 spermatids (or immature spermatozoa), ready to spermiate (Leblond and Clermont, 1952).

Sertoli cells are nongerminal cellular elements and the backbone of the seminiferous epithelium. It is known that these cells, besides playing a structurally supportive role in the germinal epithelium, are implicated in some other functions such as steroidogenesis (Lacy and Pettitt, 1970; Kerr et al. 1984) and protein synthesis (Weddington et al. 1975; Bardin 1978), both involved in the regulation of the spermatogenic process. Moreover, material derived from germ cells and phagocytized by Sertoli cells is utilized to form lipid-based products, which seem to regulate the spermatogenic process (Kerr et al. 1984).

The intimate correlation between Sertoli cell synthetic activity and spermatogenesis is controlled by hipophyseal gonadotrophic hormones FSH and LH and testosterone. Both FSH and testosterone are required for spermatogenesis acting throughout Sertoli cells. LH is also important but all its effects seem to be mediated by way of testosterone from Leydig cells (see review in Bardin, 1978).

The present investigation shows that a decrease in step 19 spermatids and Sertoli cells, observed at 24 months of age, is accompanied by a significant decrease in plasma FSH. Moreover, significantly lower levels of LH and T were observed before this time, at 12 months. Our results agree with the reports by Bruni et al. (1976), Riegle and Meites (1976) and Riegle and Miller (1978), who reported decrease FSH, LH and T levels in aged male rats.

After acute (60 min) hCG stimulation, plasma T level exhibited a significant increase in all groups of animals, although the response was smaller in 12 and 24 month groups as compared to the 3 month group (see Fig. 5). Riegle and Miller (1978) observed no age difference in serum T level between young (4 months) and aged (22 to 30 months) rats following chronic hCG stimulation (7 days), and suggested that the primary cause of reduced gonadal endocrine functions was that of inadequate gonadotrophin stimulation of testis. Riegle and Miller (1978) also compiled evidence that pituitary of aged male rats retained response capability to LHRH stimulation, and that the hypothalamus was probably a primary site of age-related alterations in the male reproductive tract.

In the present investigation, a decreased concentration of spermatozoa was observed in the cauda epididymidis at 24 months of age. The cauda of the epididymidis plays a major role in the storage of spermatozoa which have matured in the proximal regions (caput and corpus epididymidis). The decrease in spermatozoa concentration in the sperm stored in the cauda epididymidis reflected a reduced sperm production at 24 months of age, as revealed by the decrease in the amount of step 19 spermatids per seminiferous tubule cross section.

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