Scanning Electron Microscopy of Seminiferous Tubules and Spermatozoa of Rats under Conditions of Thermal Exposure

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 143, No. 1, pp. 117-120, January, 2007
Original article submitted June 20, 2006

Convoluted seminiferous tubules and spermatozoa of albino rats under conditions of thermal exposure were studied by scanning electron microscopy. Exposure at 40°C (in a thermostat) for 1 h caused significant changes in spatial structure of the tubules and spermatozoa. The tubular lumens acquired an irregular shape. The integrity of the spermatid layer was disrupted. The spermatozoon heads were deformed, the tails became bifurcated and convoluted. The appearance of erythrocytes on the spermatid surface attested to impairment of the blood-testis barrier.

Key Words: *seminiferous tubules; spermatozoa; thermal exposure*

Functional morphology of spermatozoa (SZ) is evaluated by various electron microscopic methods, including transmission and scanning electron microscopy [1,2,5-10]. Normally, SZ are not a homogeneous cell population; an appreciable part of these cells have pathological deviations. At least 30% SZ in the ejaculate of fertile men should be morphologically normal [3,4,9].

Exposure to high temperature is an important factor affecting spermatogenesis and causing morphological changes in SZ. In humans, the optimal temperature for SZ should be 1.5-2.0°C below the body temperature. The position of the testicles outside the abdominal cavity, pronounced plication of the scrotal skin, its intensive blood supply determine high heat emission and respective reduction of the scrotal temperature required for normal spermatogenesis [3,8]. Increase of temperature by even 1°C causes disorders in this process [3,8].

We studied the effects of elevated environmental temperature on SZ in rat ejaculate, particularly on SZ morphology, by scanning electron microscopy (SEM).

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MATERIALS AND METHODS

The testicles of Wistar rats (120-150 g) kept under standard vivarium conditions at 23-25°C (n=8; normal) and after 1-h exposure to 40°C in a thermostat (n=14) were studied by SEM. All males were kept in individual boxes without females. Experiments were carried out in April-May.

The preparations for evaluation of SZ morphology by SEM were processed using a method modified by us. The testicles were cut into 0.2-0.3 cm^3 fragments with a razor. The fragments were centrifuged for 3 min at 5000 rpm. A 10-fold volume of 2.5% glutaraldehyde in PBS was added to the centrifugate (volume <0.5 ml). After 1.5-h fixation the preparation in centrifuge tubes was centrifuged at 5000 rpm for 5 min. After removal of the supernatant (fixative) the precipitate was washed twice in PBS and 1% OsO_4 in the same buffer was added for 1 h. The precipitate was washed in cold 50% ethanol and placed into a fresh portion of 50% ethanol for 10 min, after which it was dehydrated in ethanol and acetone by the standard method. After dehydration in the last portion of acetone, the precipitate was fragmented, the fragments were

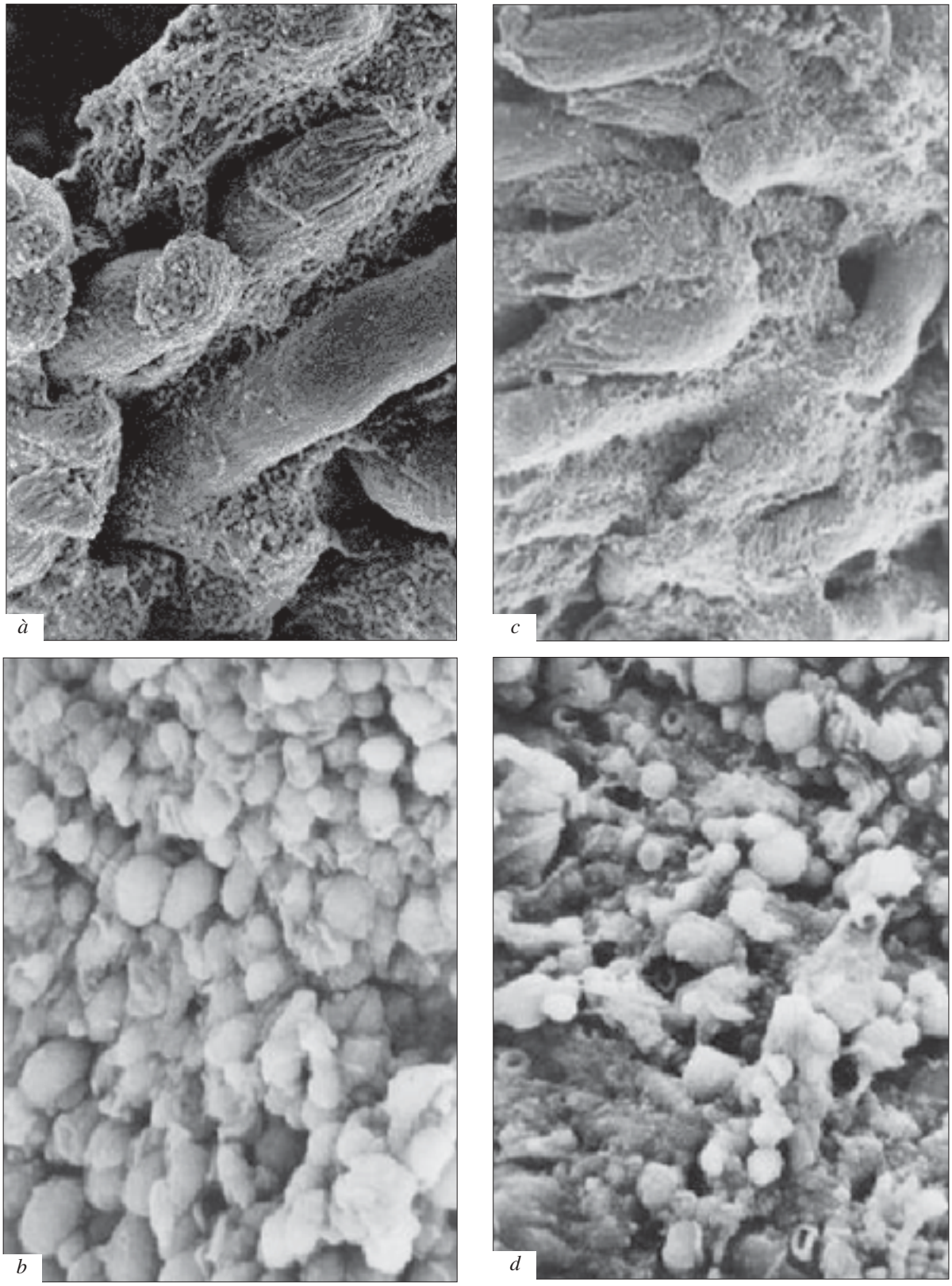

Fig. 1. Convoluted seminiferous tubules and spermatids normally (*a*, *b*) and after thermal exposure (*c*, *d*). *a*) even convoluted seminiferous tubules of a control rat, ×400; *b*) layer of round oval spermatids with even surface from a control rat, ×2000; *c*) different thickness of seminiferous tubules, ×200; *d*) impaired integrity of spermatid lining, shape, appearance of erythrocytes on their surface, ×1000.

placed into containers, and dried by the critical point method in an HCP-2 device. Dried fragments were mounted on foil with a glue conducting electric current and sprayed with gold in an IB-3 device.

For examination of tubules, the fragments were fixed and processed as described previously without centrifugation. The preparations were examined under a SEM 405A microscope (Hitachi).

RESULTS

Normally, the convoluted seminiferous tubules in rats are even tubules with fine connective tissue layers

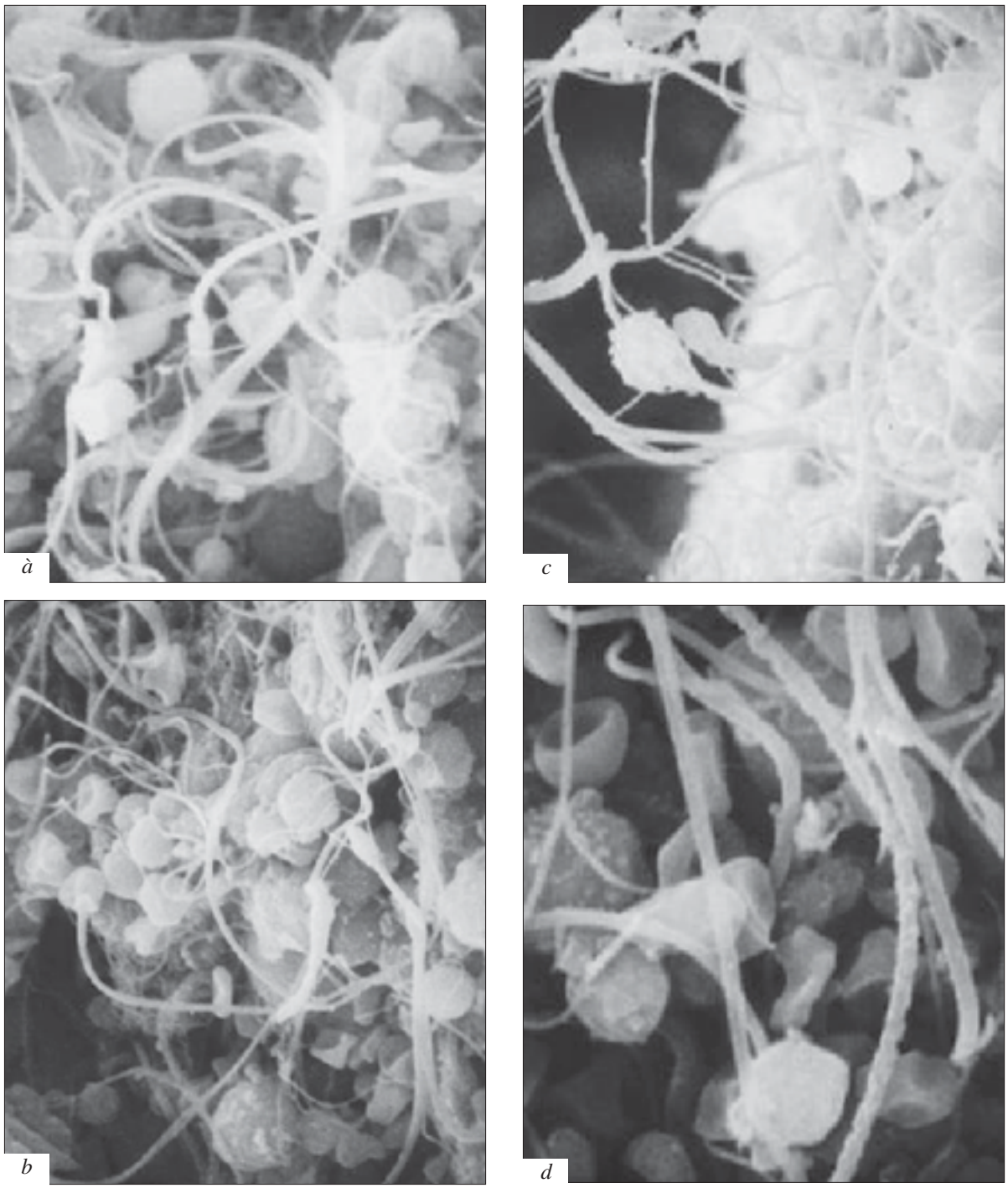

Fig. 2. Spermatozoa normally (*a*) and after thermal exposure (*b-d*). *a*) rat SZ with even heads and tails, round spermatids, ×4000; *b*) polymorphic heads and tails of rat SZ, pathological forms of erythrocytes among SZ, ×2000; *c*) scalloped surface of SZ heads and twisted tails, ×2000; *d*) SZ tails looking as ropes, ×4000.

between them. The sections or breaks of seminiferous tubules are usually oval or round (Fig. 1, *a*).

Spermatids are usually round or oval with smooth translucent surface, lying close to each other, forming an even translucent lining (Fig. 1, *b*).

One-hour exposure at 40°C caused pronounced deformation of convoluted seminiferous tubules. Their thickness becomes uneven along their length, with alternating thick and thin sites (Fig. 1, *c*). On sections and fractures, the seminiferous tubules were of irregular shape. The layer of spermatids was disrupted.

The size and shape of spermatids varied, their surface was not smooth, but had fossae and depressions. At some sites the spermatid layer was impaired and the underlying structures were denuded (Fig. 1, *d*). Erythrocytes were often seen on the surface of round spermatids, many erythrocytes had pathological shape among them stomatocytes were most incident (Fig. 1, *d*).

The presence of erythrocytes on the surface of spermatid layer indicates that thermal exposure not only induced changes in the structure of the entire layer of round spermatids, the main component of spermatogenesis, but also impaired permeability of the blood-testicle barrier.

Significant changes were also seen in SZ. Normally they have elongated flat heads with smooth surface. Their tails are thin, long, of even thickness along the entire length, and slightly twisted (Fig. 2, *a*). Exposure to high temperature resulted in polymorphism of their heads and tails by length and thickness, as well as by shape (Fig. 2, *b*). Numerous round spermatids and other spermatogenic epithelium cells were seen among SZ; the surface of these cells was uneven, with protrusions and depressions (Fig. 2, *b*).

Spermatozoa with scalloped heads and twisted tails looking like ropes appeared after exposure to high temperature (Fig. 2, *c*, *d*). Spermatozoa with thick and thin tails bifurcated in the distal portions were detected (Fig. 2, *d*). Pathological erythrocytes (echinocytes and stomatocytes) were more often seen among SZ with modified heads and tails (Fig. 2, *b*, *d*).

The appearance of erythrocytes on the surface of spermatogenic epithelium andappreciable number of their pathological forms in SZ suspension indicates not only impairment of the blood-testicle barrier permeability, but also significant transformation of erythrocytes under the effect of high temperature.

Hence, exposure to high temperature leads to significant changes in the three-dimensional ultrastructure of the seminiferous tubules and SZ. Indirect signs of impaired blood-testicle barrier were detected.

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