

MORPHOLOGICAL CHANGES IN TESTES AND EPIDIDYMIDES OF RATS AFTER GOSSYPOL

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ABSTRACT Light microscopic and electron microscopic changes were seen in testes and epididymides of rats which had become infertile after treatment with gossypol 15 mg/kg qd × 8–12 wks. Spermatogenesis was basically normal, but obvious damages consisting of vacuolization and missing mitochondria were noted in the spiral sheaths of testicular and epididymal spermatozoa. Epididymal spermatozoa were frequently found to be lacking membranes, outer fibers or inner microtubules. Cells of the epididymal epithelia showed degenerative mitochondria. A large number of blebs from dilated microvilli were noted on the surface of the epididymal ducts. The damages of Sertoli cells in some seminiferous epithelia were found in rats treated with gossypol for 12 wks.

KEY WORDS gossypol; spermatozoa; epididymis; testis; electron microscopy

Gossypol, as a poisonous constituent of cotton seed, has been studied for a long time⁽¹⁾. Recently, Chinese investigators reported that po gossypol caused infertility in men⁽²⁾. The first experiment on the antifertility effect of gossypol utilized rats. The site of action of gossypol was not in the epididymis but in the testis^(3,4). Spermatozoa, spermatids, spermatocytes and even spermatogonia were sensitive to gossypol; sensitivity depended upon the dose administered. The Leydig cells and the

epididymal epithelia remained intact⁽⁴⁾. However, low doses affected only the spermatozoa, shown by the damage of mitochondria, microtubules and membrane⁽⁵⁾. The present experiment was conducted to determine the early changes that took place in testes and epididymides of rats treated with gossypol to induce infertility.

MATERIALS AND METHODS

Male Sprague-Dawley rats of 60 d old were housed with free access to food and water under controlled light from 7 AM to 7 PM.

Gossypol acetic acid suspended in sesame oil was fed via a stomach tube to male rats at a daily dose of 15 mg/kg × 6 d a week. Ten rats were fed for 8 wks and another 10 rats were fed for 12 wks. A 3rd group of 10 rats fed with sesame oil alone served as control. The testes and epididymides from 2 rats in each group were fixed by vascular perfusion with 5% glutaraldehyde in 0.2 M s-collidine buffer at pH 7.4. After perfusion, the testes and epididymides were cut into small pieces and further fixed in 5% glutaraldehyde, washed with s-collidine buffer, postfixed in 1% s-collidine buffered osmium tetroxide solution, dehydrated in ethanol and propylene oxide, and embedded in epon 812. Thin sections were stained with lead citrate and uranyl acetate and examined with JEM

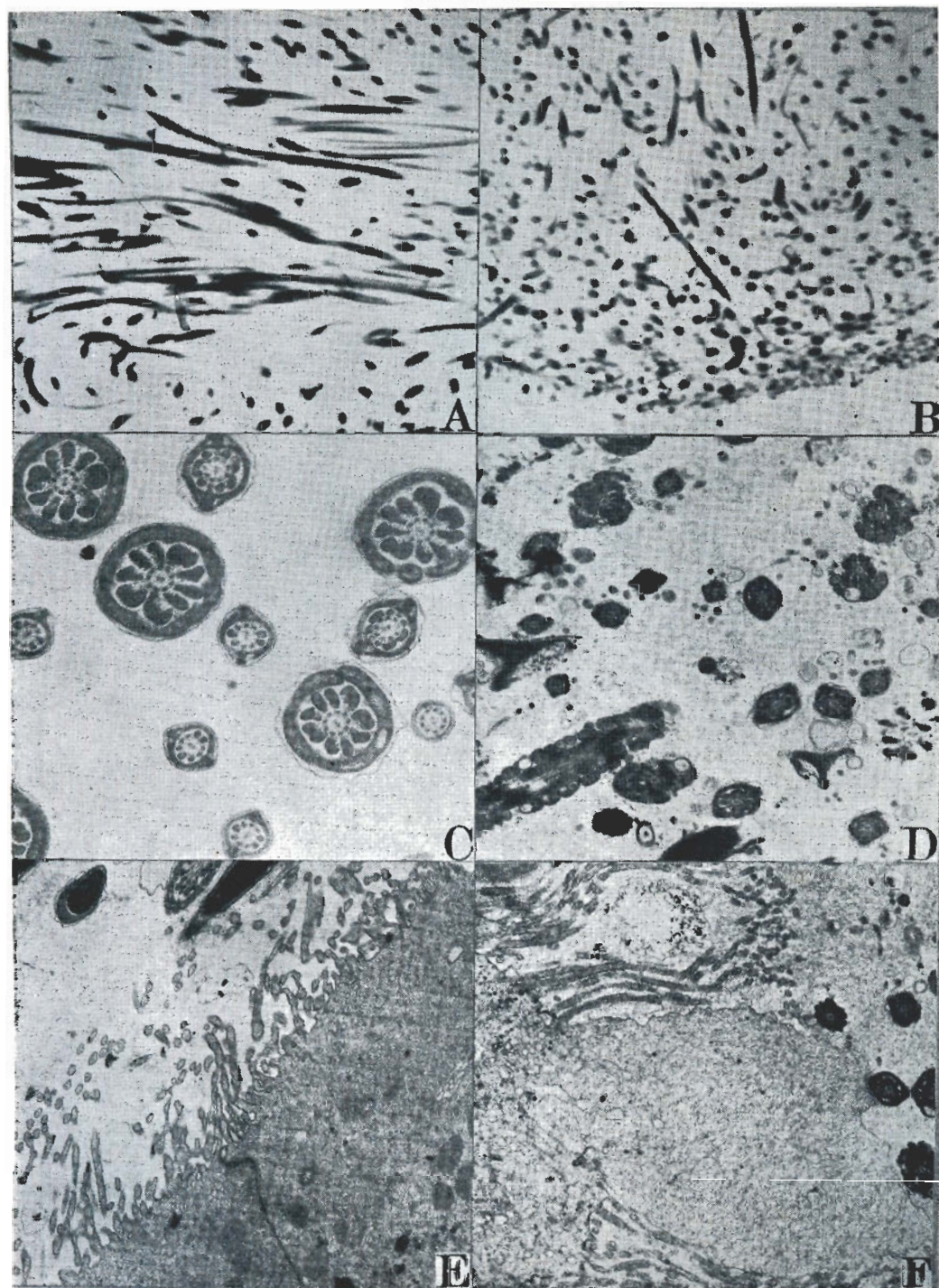
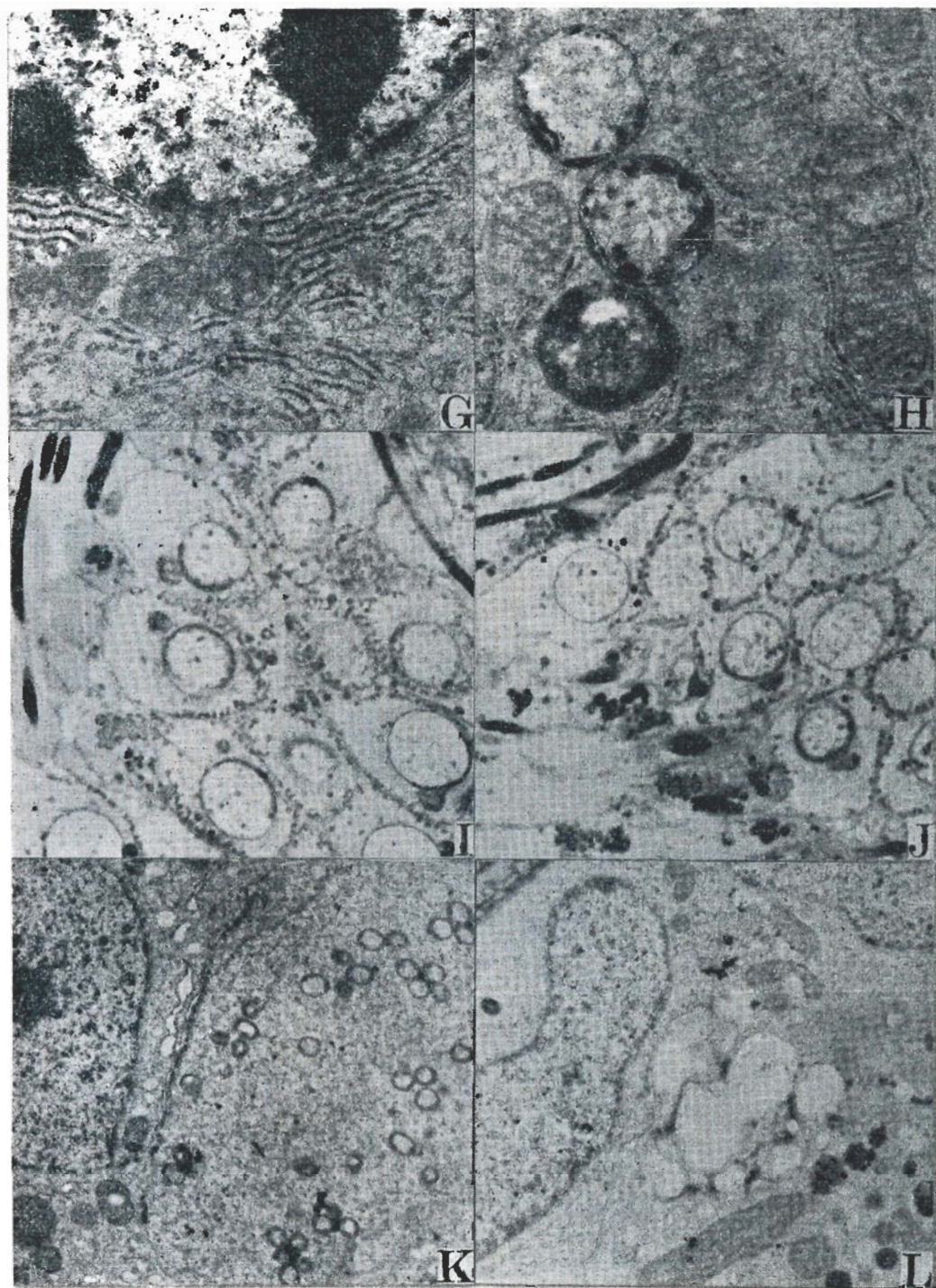


Fig 1. Control rats (A, C, E, G, I, K). Rats fed gossypol for 8 wks (B, D, F, H, J) or 12 wks (L). A, B) Epididymal spermatozoa $\times 1700$. C, D) Epididymal spermatozoa $\times 30000$. E, F) The luminal cell surface of an epididymal duct $\times 21000$.



G, H) Epididymal epithelium $\times 25000$. I, J) Testis $\times 2000$. K, L) Sertoli cells of testis $\times 21000$.

200 B electron microscope. For light microscopy, 1 μ m sections were cut and stained with toluidine blue.

RESULTS

There were no pregnancies among the 20 of female rats mated with males which had been fed with gossypol 15 mg/kg qd \times 8—12 wks. Spermatogenesis still occurred in the treated rats, but the total number of epididymal sperm was less than half of that found in the control group and most of these spermatozoa were nonmotile. In some spermatozoa, there were complete separations of the heads and tails. The number of suffered spermatozoa was greater in the group treated for 12 wks than that in the group treated for 8 wks.

Under light microscope, longitudinal sections of epididymal spermatozoa from control rats showed a smooth, continuous surface and a regular arrangement of mitochondria in the middle piece. Epididymal spermatozoa from treated rats showed a fragmented surface (Fig 1 A, B).

Under electron microscope, the mitochondria of spermatozoa in the epididymides appeared to be swollen and transparent, resulting in a loss of the uniform size and regular arrangement of the spiral sheath. In some spermatozoa, the cell membranes, mitochondrial sheath and fibrous sheath in the principal piece disappeared totally (Fig 1 C, D).

In control preparations, the epithelial cells of epididymal ducts presented microvilli as long cytoplasmic projections on the luminal cell surface; each microvillus showed a plasma membrane and a dense cytoplasmic core which seldom extended to bleb. However, the treated epithelial cells always had many large blebs on its surface as a result of the dilatation of the microvilli (Fig 1 E, F). The principal cells of epididymal ducts in control rats always showed many well developed cell or-

ganelles including large amounts of round or ovoid mitochondria with numerous plate-like cristae, and very extensive endoplasmic reticulum, especially the rough type. In contrast with the control, some of the principal cells in the epididymides of treated rats exhibited transparent vesicles and dense bodies under low magnification. Under high magnifications, both transparent vesicles and dense bodies displayed double limiting membranes with cristae-like structures inside and some pycnotic materials attached on the inner membrane. These unusual structures were apparently degenerated or damaged mitochondria (Fig 1 G, H).

Light microscopy of testes showed that the spermatogenesis was not inhibited by gossypol treatment although the thickness of seminiferous epithelium was slightly thinner than that of control rats. Spermatogonia and spermatocytes were in different phases of mitosis and meiosis respectively and spermatides were in various steps of spermiogenesis, but spermatozoa lacked the smooth, continuous surface of the tail, similar to that seen in the epididymis. Electron microscopy showed no obvious changes in the germ cells until they reached the late spermatid stage (Fig 1 I, J). In the late spermatids, the evident change was in their mitochondrial sheath, especially in their middle piece. The mitochondria underwent vacuolization and lost their cristae and dense ground substance between cristae. Because of the complete degeneration of some mitochondria, the mitochondrial sheath of spermatozoa lost its regular, continuous configuration and became fragmented. Although the mitochondria were seriously damaged in some late spermatids, the other structures of spermatids remained basically normal. For example, the 9 pairs of microtubules, the 9 pairs of coarse fibers and the cell membranes were essentially normal.

In some seminiferous epithelia of rats treated with gossypol for 12 wks, the cytoplasm of Sertoli cells became denser and shrunk, some intercellular spaces were widened, and lacuna on the cell surface were seen between the Sertoli cells. Some tight junction between Sertoli cells were broken and appeared as a fragmentary structure around empty spaces (Fig 1 K, L).

DISCUSSION

Gossypol may affect different stages of spermatogenesis depending on the dose administered^(2,4). Spermatids manifested most of the morphological damages^(4,6). In this experiment, treatment of rats with gossypol (15 mg/kg/d) qd × 8–12 wks resulted in damages to the mitochondrial sheath in late spermatids; other testicular cells appeared to be normal. Abnormalities of the configuration of testicular and epididymal spermatozoa were detected in plastic sections by light microscopy. This technique may be useful in screening the new analogues which affect the late stages of spermatogenesis (spermatids and spermatozoa).

Nadakevukaren et al, studying the effect of gossypol on epididymal spermatozoa in rats, found that the major abnormality was in the motor apparatus of the spermatozoal tail. They saw disorganization of the mitochondrial sheath, vacuolization of mitochondria, broken cell membranes and large segments of the middle-piece completely lacking a plasma membrane. They also noted that the outer fibers and inner microtubules were missing in spermatozoa of treated rats. Therefore, they suggested that low concentration of gossypol reduced the spermatozoal motility, which was responsible for its contraceptive action⁽⁵⁾. We found degenerative mitochondria almost all epididymal spermatozoa. Broken or missing membranes, missing outer fibers and inner

microtubules were found in some spermatozoa. However, in the testes, the mitochondria were seriously damaged in some late spermatids while the other structures remained essentially unchanged at 15 mg/kg/d qd × 8 wks. It is possible that gossypol interferes with the structures development of spermatozoal mitochondria in the testis and other structures later when the spermatozoa migrate to the epididymis or during maturation of the spermatozoa in the epididymis. The mitochondria of spermatozoa and late spermatids in the testis seen to be the targets most sensitive to gossypol. These morphological changes are consistent with the finding that gossypol inhibits lactate dehydrogenase X (LDH-X) located in mitochondria of spermatogenic cells and spermatozoa⁽⁷⁾. Moreover, it has been known that the specially differentiated tight junction play a significant role for seminiferous epithelium to establish the testis barrier which protects normal spermatogenesis from different extratubular influences. It is possible that the damage of tight junction could be one of the antispermatogenesis mechanisms of gossypol.

Gossypol has no effect on the epithelial cells of the epididymis⁽⁴⁾. In this experiment, however, it was found that gossypol also damaged the mitochondria of the epithelial cells in epididymal duct and induced formation of large blebs on the cell surface as a result of dilatation of the microvilli. These changes may provide an abnormal milieu to aggravate the damage of spermatozoa when they move from the caput to the cauda epididymidis.

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REFERENCES

1 Breardi CC, Goldblatt LH. Gossypol. In:

- Liener IE, ed. *Toxic constituent of plant food-stuffs*. 1st ed. NY: Academic Press, 1969:211-55
- 2 National Coordinating Group on the Male Antifertility Agents. *Chin Med J* 1978 Nov; 4 (6):417
- 3 Dai RX, Dong RH. *Acta Biol Exp Sin* 1978 Aug; 11 (1):15
- 4 Xue SP, Zong SP, Su SY, Wu YW, Liu Y, Zhou ZG, Ma XX. *Sci Sin* 1980 May; 23 (5):642
- 5 Nadakavukaren MJ, Sorensen RH, Tone JN. *Cell Tissue Res* 1979 Dec; 204 (2):293
- 6 Dai RX, Pang SW, Liu ZL. *Acta Biol Exp Sin* 1978 Aug; 11 (1):27
- 7 Maugh TH II. *Science* 1981 Apr 17; 212 (4492):314