

ラットにおける誘起潜伏精巢の組織学的変化

誌名	琉球大学農学部学術報告 = The science bulletin of the College of Agriculture, University of the Ryukyus
ISSN	03704246
著者名	仲田,正 永井,利絵 水町,進 大倉,信彦 上地,俊徳 建本,秀樹 小尾,岳士 川本,康博
発行元	琉球大学農学部
巻/号	56号
掲載ページ	p. 43-47
発行年月	2009年12月

農林水産省 農林水産技術会議事務局筑波産学連携支援センター
Tsukuba Business-Academia Cooperation Support Center, Agriculture, Forestry and Fisheries Research Council
Secretariat



ラットにおける誘起潜伏精巣の組織学的変化

仲田 正¹, 永井 利絵¹, 水町 進¹, 大倉信彦², 上地俊徳¹, 建本秀樹¹, 小尾岳士³, 川本康博¹

¹ 琉球大学農学部, ² 琉球大学医学部, ³ 鹿児島大学農学部獣医学科

Effects of the Induced Cryptorchidism on Rat Testicular Histology

T. Nakada¹, R. Nagai¹, S. Mizumachi¹, N. Ookura², S. Uechi¹⁾, H. Tatemoto¹, T. Obi³, H. Kawamoto¹

¹ Faculty of Agriculture, University of the Ryukyus, Nishihara-cho, Okinawa, Japan;

² Faculty of Medicine, University of the Ryukyus;

³ Faculty of Agriculture, Department of Veterinary Science, Kagoshima University, Japan.

ラット精巣に対する温度処理が精子形成に如何なる影響を及ぼすかを調べるため, 精巣の場所である陰嚢より1 ~8 °C高い温度下にある腹腔へ精巣を人為的に押し込み, 結紮によって精巣の脱出を防いで腹腔内停留精巣を作成した。停留処置後5日, 10日および15日目に精巣を摘出し, 組織標本作製して精巣精細管における組織像を観察した。精巣の停留処置を行わなかった片側の陰嚢内精巣は対照として用いた。

その結果, 精巣停留処置後5日目の精細管で, すでに各種精細胞に退行的変化が観察され, 特にその変化は精母細胞, 精娘細胞, 精子細胞および精子に出現した。また, 精細胞の変化に伴って食食作用を有する巨大多核細胞が出現した。精細胞における退行変化は精巣の腹腔内停留期間が延長するにつれ顕著になり, 処置後15日目の精細管では精祖細胞以外の精細胞は完全に消失した。同様な退行的変化は精子の通路である精巣上体管においても観察された。処置後5日目で精巣上体内精子集落に巨大多核細胞が出現し, 処置後10日では精子集落が赤色球形細胞に変化し, 15日目では精巣上体管は精細胞を欠き, その内腔は空白を呈した。

Abstract

Unilateral cryptorchidism was induced in adult rats for 5, 10 and 15 days and its influence on testicular morphology was investigated. In seminiferous tubules from abdominal testes the degenerative changes of germ cells was manifested in spermatocytes, spermatids and spermatozoa in Day 5 after the induction of cryptorchidism.. These germ cells were almost absent by 15 days after the induction of cryptorchidism. On the other hand, spermatogonia and Sertoli cells were maintained in the failure of some morphological damages during experimental cryptorchidism. It is suggested that spermatocytes and young spermatids like spermatocytes, spermatids and spermatozoa were the most sensitive germ cells to body heat, whereas spermatogonia and Sertoli cells showed strong resistance to it.

It was thought that multinucleated giant cells appeared at early time following artificial cryptorchidism might be responsible for the elimination of degenerated germ cells through phagocytosis by the cells.

キーワード; 潜伏精巣, 精子形成, 精巣上体, 陰嚢, 多核巨大細胞

Key words: cryptorchidism, spermatogenesis, epididymis, scrotum. multinucleated giant cells.

Introduction

In most mammals, including humans, the testes descend into scrotum by birth or shortly thereafter. Sometimes the testes retained in the abdominal cavity and failed to enter the scrotum. Such a condition is called a cryptorchidism. Cryptorchidism is associated with either greatly reduced or absent spermatogenesis (Albescu et al., 1971; Cummins and Glover, 1970; Davis and Firlit, 1966; Giarola, 1967).

In addition to serving as a covering for the testes, the scrotum functions as a thermoregulatory mechanism. This function of the scrotum is manifested by actual differences in temperature, the temperature in the scrotum being from 1 °C to 8°C lower than that of the abdominal cavity. It has been shown by several workers that insultation or the application of the heat to the testes resulted in a degeneration of the spermatogenetic tissue, the production of abnormal sperm, and temporary sterility (Kandeel and Swerdloff, 1988; Chowdhury and Steinberger, 1964; Collins and Lacy, 1969). However, why

high temperatures are injurious to spermatogenesis is not known.

In this experiment, using the induced cryptorchid rats, the histological changes of germ cells in seminiferous tubules was investigated concerning when or which germ cells are affected after cryptorchidism.

Materials and Methods

Animals: Ten adult (80~90-day-old) male Sprague-Dawley rats (350~375g) purchased from Japan Charles River company, Tokyo Japan, were used in this experiment. Animals were housed in a standard animal facility under controlled temperature (22°C) and photoperiod (12 h of light, 12 h of darkness) with free access to water and rat chow throughout the experiment.

Induction of cryptorchidism: To render the animals unilaterally cryptorchidism, either the left or right testis was manually moved into the abdominal cavity through the inguinal canal and ligated by thread through the abdominal wall so that the testis could not re-enter the scrotum. Unilateral cryptorchid testis and its epididymis were excised from them after killing with ether anesthesia at 5, 10 and 15 days after the induction of cryptorchidism. Contralateral testis undescended into abdomen and epididymis were also excised at the same time. The contralateral undescended testis were used as controls and processed in the same way as the cryptorchid testis.

Staining: Testes and epididymis were fixed with formaldehyde which diluted with water (10%) and embedded in paraffin wax. These specimens were sectioned at a thickness of 8μm, stained with haematoxylin-eosin and examined histologically under light microscope.

Results

Testes histology: Throughout 5, 10 and 15 days after the induction of cryptorchidism, the epithelial lining of spermatogonia, spermatocytes, spermatids and spermatozoa in the seminiferous tubules of the contralateral undescended testis (control) were normal (Fig. 1a). All the stages of the cycle of the epithelial germ cells were arranged regularly. Multinucleated giant cells were never observed. Sertoli cells could seen with normal feature were along the wall of seminiferous tubules (Fig. 1b). The lumen of epididymis was filled with the colony of spermatozoa (Fig. 2a).

In the 5-day cryptorchid testis (Fig. 1c,d) alteration of the germinal epithelium in the seminiferous tubules was manifested by irregular arrangements of epithelial cells. In some seminiferous tubules, germ cells except for spermatogonia disappeared and there was a reduced diameter and disorganization of spermatocytes, spermatids and spermatozoa

in some seminiferous tubules. Multinucleated giant cells were seen as large rounded structure. At higher magnification, it was shown that these cells contained a large number of germ cells in it. In epididymis (Fig. 2b, c), the appearance of red round cells and a few binucleated cells were observed. The size of the spermatozoal colony was reduced and that space between the tubal wall and the colony was formed. In the 10-day cryptorchid testis (Fig. 1e,f), a large of seminiferous tubules were lacked in germ cells other than spermatogonia and Sertoli cells. In a few tubules, binucleated cells concentrated in the center of the lumen. In epididymis (Fig. 2d,e), spermatozoa was almost absent, and the colony of spermatozoa almost changed to red round cells. In the 15-day cryptorchid testis (Fig. 1g,h), most germ cells such as spermatocytes, spermatids and spermatozoa lost but spermatogonia and Sertoli cells were still present in the majority of seminiferous tubules. In these epididymis (Fig. 2f), spermatozoa were completely devoided for which the epididymal lumen was founded empty.

Discussion

Experimental cryptorchidism is a common model for examining the expression and function of heat-sensitive spermatogenesis in testes. In most mammals, including humans, the testes are always maintained at a lower temperature than that in the abdomen. An exposure of the testes to abdominal temperature or above results in increased death of germ cells (Nalbandov, 1976). When testes were immersed for 15 minutes in bath maintained at 43°C, testicular DNA fragmentation showing the apoptotic nature of germ cells death was observed within 1 and 2 days after heat treatment.

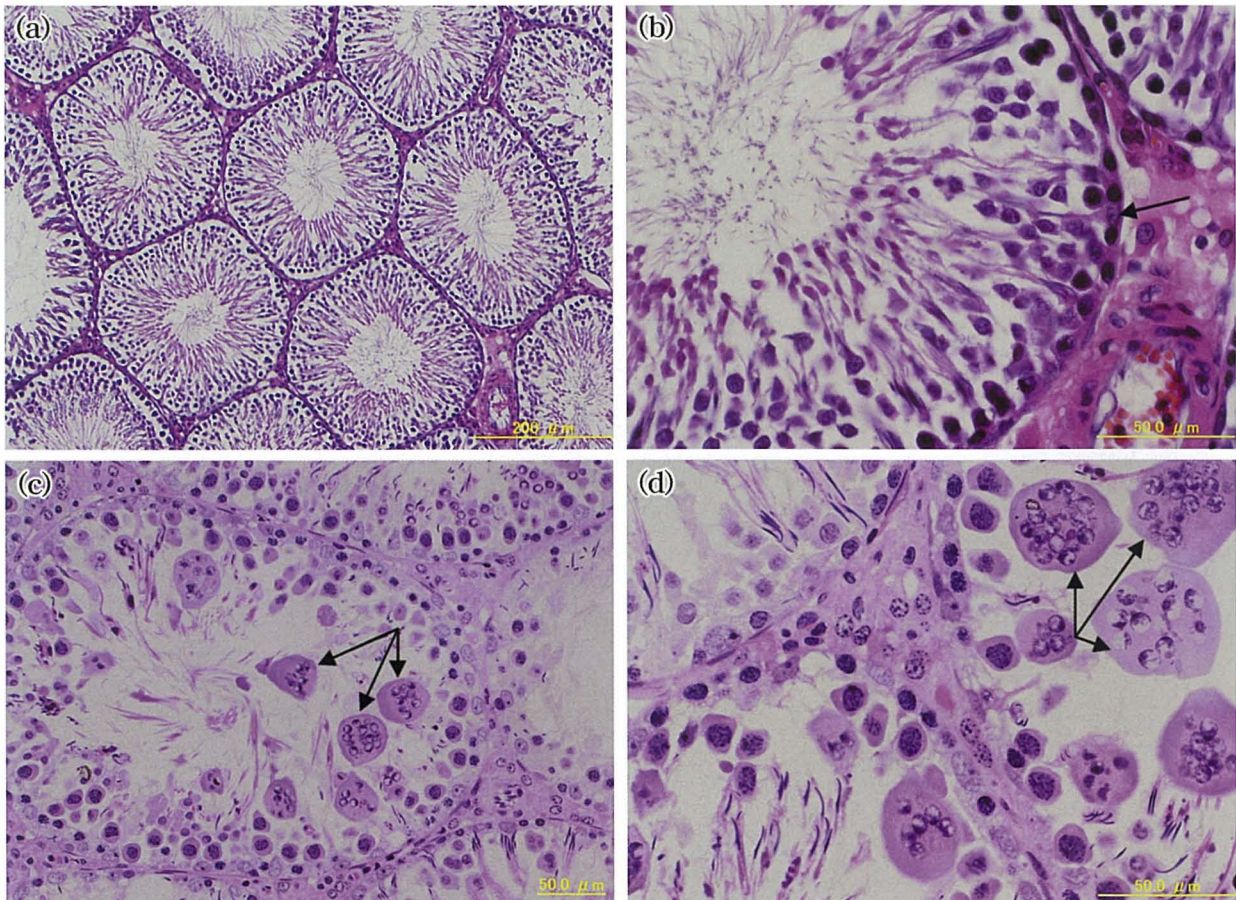
In the 5-day after the induction of cryptorchidism, some damages to germ cells of abdominal testis was manifested by the reduction of a few germ cells and the appearance of multinucleated giant cells, whereas such a degenerative changes in spermatogenesis were not observed in control. Some damages to germ cells were limited to spermatocytes, spermatids and spermatozoa, and Sertoli cells remained unaffected. Similar results have been reported by Jone et al. (1977) that the degenerative changes of germ cells could be seen in the spermatids and spermatocytes within 24 hours of transfer of the testis to the abdominal cavity. It suggests that spermatocytes, spermatids and spermatozoa were most susceptible to heat.

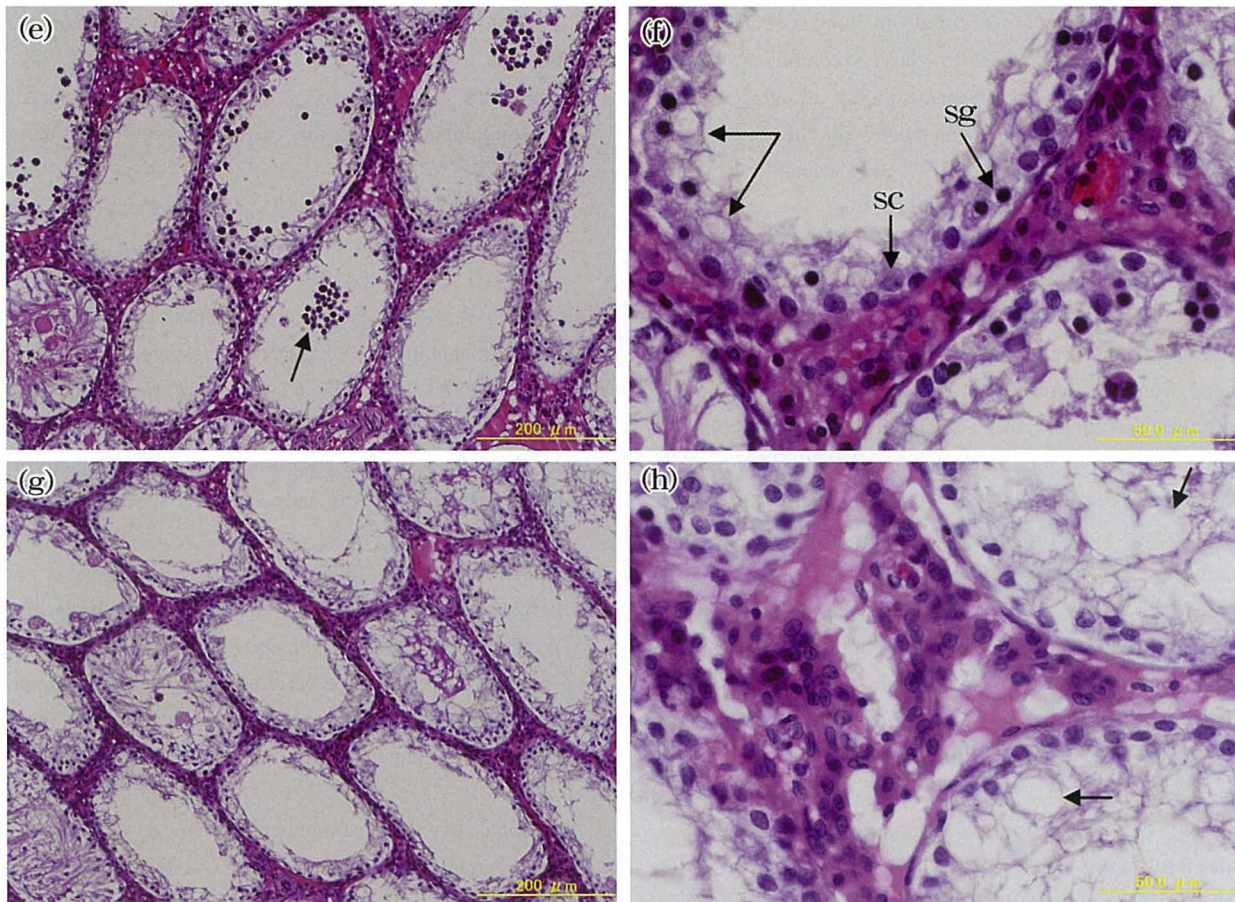
The extent of germinal epithelium disruption increased according to the time that had elapsed after artificial cryptorchidism. In the 15-days after the induction of cryptorchidism the marked loss of germ cells other than spermatogonia and Sertoli cells were displayed in most seminiferous tubules. Spermatogonia was morphologically unaffected during the full length of the experiment period,

however, it appeared that it could no longer differentiate beyond the spermatocyte stage by the cryptorchidism because germ cells originating from spermatogonia diminished these numbers and disappeared in accompany with the extension of cryptorchidism. It suggested that the capability in the differentiation of spermatogonia rather than the morphology might be affected by cryptorchidism. The appearance of multinucleated giant cells containing a few germ cells among them was closely related to the decrease of germ cells in the seminiferous tubules; presumably most of germ cells were eliminated from the seminiferous tubules through phagocytosis by multinucleated giant cells. Although the Sertoli cells has also phagocytosis like multinucleated giant cells, however, the relationship between them in the phagocytosis was remained unknown in this experiment. It was interesting to know in cryptorchid testis that the spermatozoal colony in the epididymis have transformed the shape to the colony composing of red round cells once before vanishing from the epididymal lumen.

References

- 1) Albescu, R., J. Zamudio, C. Bergada, M. Gullen: 1971. Male fertility in patients treated for cryptorchidism before puberty. *Fertil. and Steril.*, 22: 829-883
- 2) Chowdhury, A, K., E. Steinberger: 1964. The influence of a cryptorchid milieu on the initiation of spermatogenesis in the rat. *J. Reprod. Fertil.*, 29: 173-178
- 3) Cummins, J. M., T. D. Glover: 1970. Artificial cryptorchidism and fertility in the rabbit. *J. Reprod. Fertil.*, 23: 423-433
- 4) Davis, J. R., C. F. Firlit: 1966. The germinal epithelium of cryptorchid testes experimentally induced in prepubertal and adult rats. *Fertil. and Steril.*, 17: 187-200
- 5) Giarola, A. 1967. Protection of reproductive capacity as a factor in therapy for undescended testicle. *Fertil and Steril.*, 18: 375-380
- 6) Jones, T. M., W. Anderson, V. S. Fang, R. L. Landau, R. L. Rosenfield: 1977. Experimental cryptorchidism in adult male rats: Histological and hormonal sequelae. *Anat. Rec.*, 189: 1-27
- 7) Nalbandov, A.V. : 1976. The male reproductive system. In: *Reproductive physiology of mammals and birds*. 3rd ed., W. H. Freeman and Company San Francisco, pp38-45.
- 8) Kandeel, F. R., R. S. Swerdloff: 1988. Role of temperature in regulation of spermatogenesis and the use of heating as a method for contraception. *Fertil. and Steril.*, 49: 1-23
- 9) Collins, P., D. Lacy, : 1969. Studies on the structure and function of the mammalian testis, II. Cytological and histochemical observations on the testis of the rat after a single exposure to heat applied for different lengths of time. *Proc. R. Soc. Lond [B]* 172: 17-23

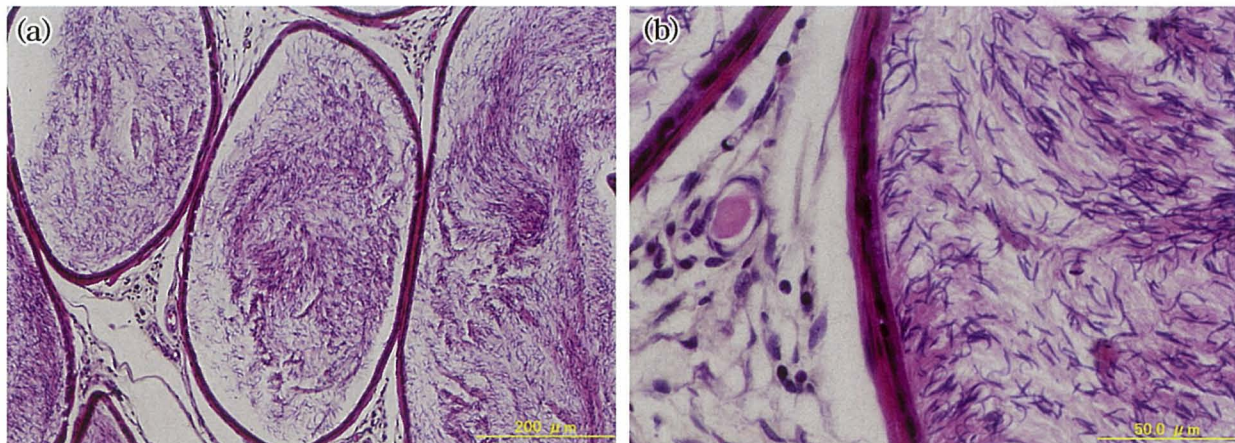




Explanation of Figures

Fig. 1. Cryptorchid rat testes stained with Haematoxylin-eosin. (a) In the contralateral testis (control) at 5 days after the induction of cryptorchidism of lateral testis, germinal cells composing of spermatogonia, spermatocytes, spermatids and spermatozoa are lined with regular sequence in the seminiferous tubules. Sertoli cells(asterisks) are observed under the wall of the tubules. x 100. (b) At high magnification(x 400)showing normal arrangement of germinal cells and Sertoli cells(arrow). (c) Seminiferous tubule from an unilateral cryptorchid rat testis 5 days after the induction of cryptorchidism. Multinucleated giant cells appears as large rounded structure within the tubules(arrows). x 200.

(d) High magnification(x 400) shows multinucleated giant cells containing a large of germ cells in it(arrows). (e) Seminiferous tubules at 10 days after the induction of unilateral cryptorchidism. Tubules showing disappearance of germ cells and enlargement of the lumen. Sloughed cells concentrate in the lumen or exist near the basal part of the seminiferous tubules(arrow). x 100. (f) High magnification showing a marked vacuolization (arrows). sc; Sertoli cell, sg; spermatogonia. x 400. (g) Seminiferous tubules from an unilateral cryptorchid rat testis at 15 days after the induction of cryptorchidism. Most epithelial cells but for spermatogonia and Sertoli cells degenerate in the tubule. x 100. (h) High magnification showing a marked vacuolization (arrows). x 400.



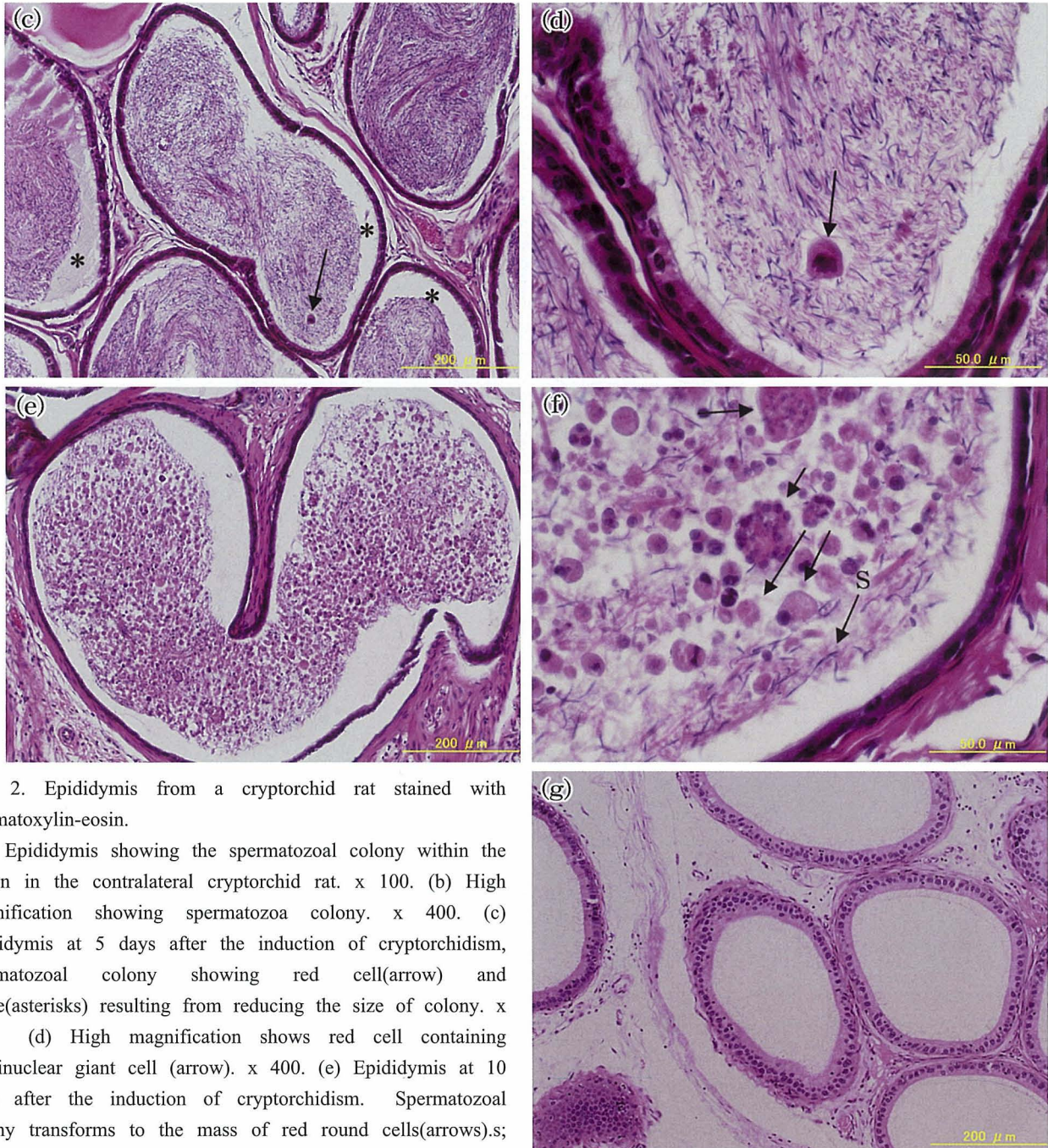


Fig. 2. Epididymis from a cryptorchid rat stained with Haematoxylin-eosin.

(a) Epididymis showing the spermatozoal colony within the lumen in the contralateral cryptorchid rat. x 100. (b) High magnification showing spermatozoa colony. x 400. (c) Epididymis at 5 days after the induction of cryptorchidism, spermatozoal colony showing red cell (arrow) and space (asterisks) resulting from reducing the size of colony. x 100. (d) High magnification shows red cell containing multinuclear giant cell (arrow). x 400. (e) Epididymis at 10 days after the induction of cryptorchidism. Spermatozoal colony transforms to the mass of red round cells (arrows); spermatozoa. x 100. (f) High magnification showing a few residual spermatozoa (arrows). x 400. (g) Epididymis at 15 days after the induction of cryptorchidism. Lumen shows empty without spermatozoa. x 100.