

동결보존된 생쥐 고환조직 세포의 광학 및 전자현미경적 관찰

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Light and Electron Microscopic Observation in the Frozen-thawed Mouse Testicular Tissues

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Objective: The aim of this study was to investigate the morphological aspects of testicular tissue before and after freezing-thawing by light and transmission electron microscopy.

Methods: Tissue biopsies were carried out on mouse testis for freezing. Samples in medium containing 20% glycerol were frozen by computer-controlled freezing program. The effect of freezing-thawing on the structural change of testicular tissues were examined by light and electron microscopy.

Results: The freezing-thawing procedure had no significant effect on tubular diameter. However, it caused folding of the lamina propria, and notable damage to Sertoli cells, spermatogonia and spermatocytes. The cells were detached, desquamated from the basal lamina and had increased vacuolization. Round spermatids, elongated spermatids and spermatozoa were less affected, and most of them maintained their normal structure.

Conclusions: The structure of spermatogonia, spermatocyte and basal compartments in seminiferous epithelium was significantly altered by freezing-thawing procedure of mouse testicular tissues. Thus, we need to develop a more reliable method for the cryopreservation of testicular tissues.

Key Words: Freezing-thawing, Ultrastructure, Testicular tissues, Seminiferous tubule

1-5
IVF-ET
가

가 . 2.

6-10 computer-controlled freezing program
cryovial 20
4 -0.5 /min , -10 /
min -90 LN₂
vial LN₂ , 1
37 10 .

가 2 0.4% HSA가 Ham's F-10

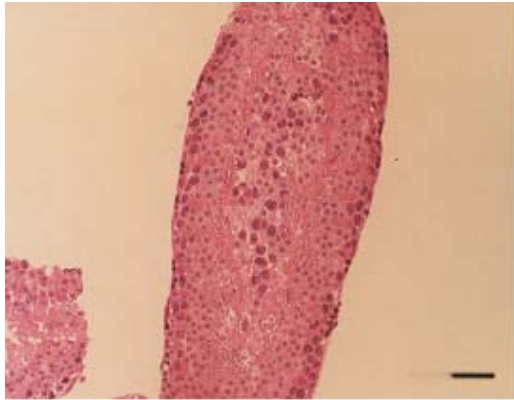
가 3.

가 glycerol, DMSO (di-
methyl sulfoxide) . Glycerol
phosphate buffer
pH
가
, DMSO glycerol 50%
xylene
7~8 μm hema-
11.12 toxylin eosin

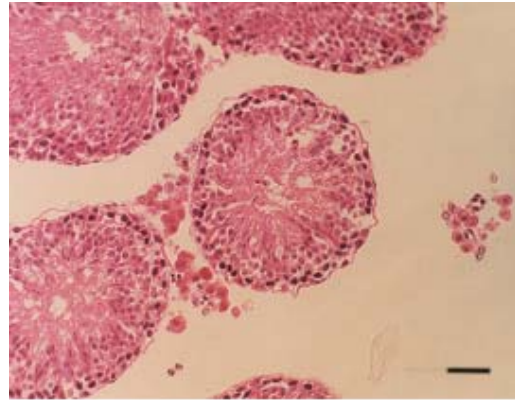
IVF-ET
glycerol 4.

glycerol
0.1 M phosphate
buffer (pH 7.2) 4% glutaraldehyde 1 ml
10 1 mm³
0.1 M phosphate buffer
(pH 7.2) 1% OsO₄ 90
1. 50%

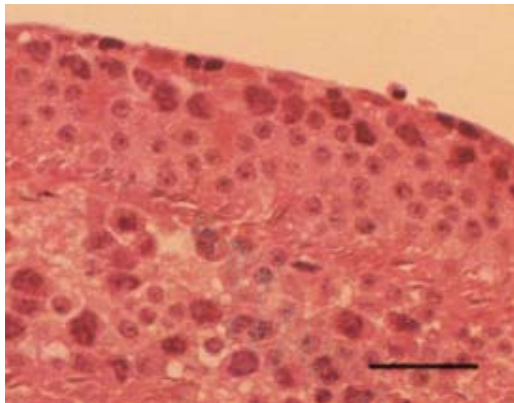
10 propylene
0.4% HSA가 가 Ham's F-10/HEP- propylene : epon (1 : 1) 12
ES Epon 812
DMEM/F-12 20% glycerol 가



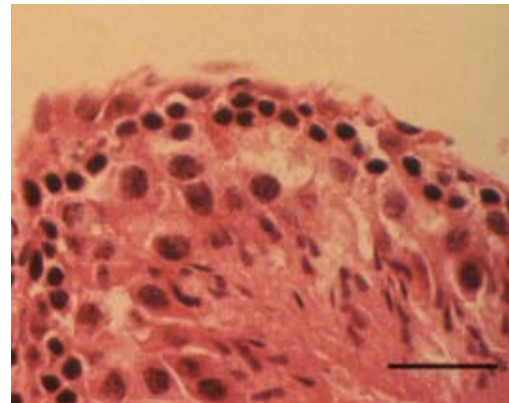
A



B



C



D

Figure 1. Light micrographs of seminiferous tubules. (A, and C) Seminiferous tubule before the freezing-thawing procedure. The tubule is surrounded by the gentle lamina propria within the basal tubular compartment, spermatogonia and the major part of Sertoli cells. In the apical tubular compartment spermatocytes and round and elongated spermatids are visible. (B, and D) Seminiferous tubule after the freezing-thawing procedure. Within the seminiferous epithelium, the 'gap' is extended from the basal to the apical compartment. Some spermatogonia and Sertoli cells are detached from the basement membrane. Sertoli cell cytoplasm is displayed many vacuoles of different size. Most of the cells in basal compartment are shrunken and divided from the apical part of the seminiferous epithelium by gaps. Round and elongated spermatids seem to be mostly preserved (Bar = 50 μ m).

5. 75 nm
 - copper grids uranyl acetate lead
 propria, lamina citrate lamina
 , Sertoli cell propria, spermatogonia, spermatocyte, spermatid, sperma-
 . spermatogonia tozoa ,
 . Equatorial segments
6. postacrosomal region
 Diamond knife elongated spermatids spermatozoa .

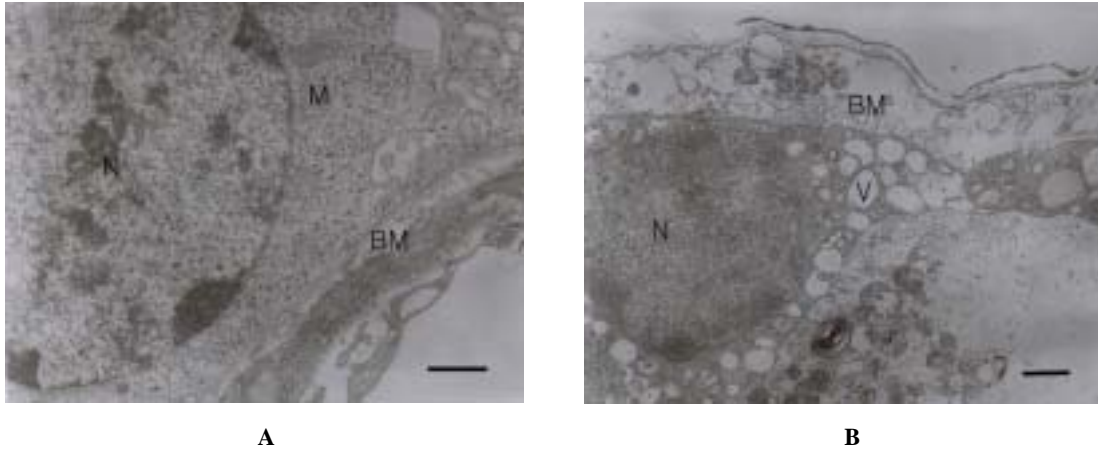


Figure 2. Electron micrographs of spermatogonia and basement membrane. **(A)** Spermatogonia and basement membrane before the freezing-thawing procedure. The tubular lamina propria is consisted of one layer of gentle peritubular (myoid) cells facing the basement membrane of the seminiferous epithelium. Spermatogonia are located on the basal lamina of the tubule. **(B)** Spermatogonia and basement membrane after the freezing-thawing procedure. Spermatogonia shows detachment, desquamation from the basement membrane, and increased vacuolization. N = nucleus; M = mitochondria; V = vacuole; BM = basement membrane; Bar = 1 µm

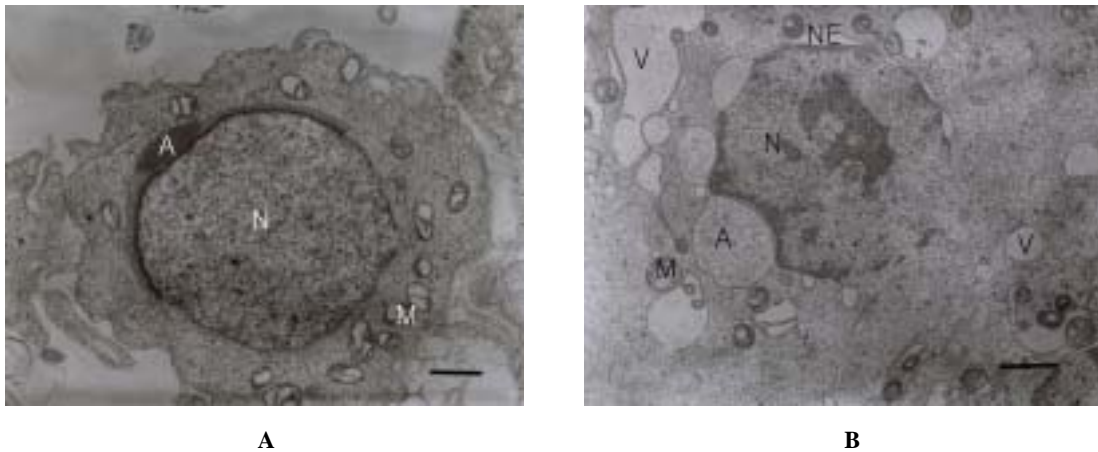
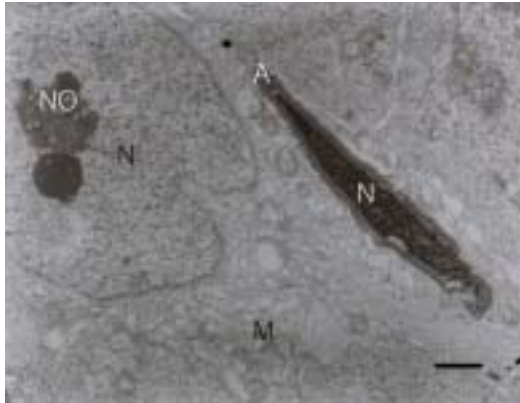


Figure 3. Electron micrographs of early-stage spermatids. **(A)** Early-stage spermatid before the freezing-thawing procedure. The nuclear envelope is thickened over the whole area of the contact with the acrosomal vesicle by a thin electron-dense layer of perinuclear theca. The acrosomal granule is present as a small electron-dense mass. Mitochondria are found at the cell periphery. Endoplasmic reticulum are visible in the cytoplasm, close to electron-dense granules. **(B)** Early-stage spermatid after the freezing-thawing procedure. The nucleus clumping of chromatin is observed which suggests a degenerative process. Nuclear membrane is disrupted and swollen. The acrosomal granule is not visualized in this cell. A = acrosome; N = nucleus; NE = nuclear envelope; M = mitochondria; V = vacuole; Bar = 1 µm

1. Lamina propria
 lamina propria inner layer germinal epithelium basal layer
 Sertoli cell



A



B

Figure 4. Electron micrographs of spermatozoa. **(A)** Spermatozoa before the freezing-thawing procedure. The chromatin is fully condensed. The acrosomal matrix is clearly present covering most of the sperm head. **(B)** Spermatozoa after freezing-thawing procedure. The inner acrosomal membrane, outer acrosomal membrane and plasma membrane are swollen. A = acrosome; N = nucleus; NO = nucleolus; M = mitochondria; V = vacuole; Bar = 1 μ m

가
 spermatogonia, spermatocytes, early spermatids
 가
 Late spermatids spermatozoa seminiferous epithelium

(Figure 1).

2.

1) Lamina propria

seminiferous epithelium basement membrane peritubular cells (myoid)가
 - lamina propria가 folding
 (Figure 2).

2) Sertoli cells

Sertoli cell seminiferous epithelium basement membrane
 Sertoli cell basement membrane

3) Spermatogenic cells
 spermatogenic cell

Spermatogonia basement membrane basal tubular compartment
 Spermatocytes round, elongated spermatids lumen
 Sertoli cell

basal compartment, spermatogonia Sertoli cell

Spermatogonia basement membrane 가
 Sertoli cell spermatogonia basement membrane lamina propria

(Figure 2). Spermatocyte basal tubular compartment, , , spermatogonia
 Round spermatids elongated spermatids - round spermatids

(Figure 3). Elongated spermatids/spermatozoa 가 , 가 가 . Elon-

gated spermatids/spermatozoa spermatogonia spermatocyte 가 . Spermatozoa

/ . Spermatozoa 가

(Figure 4).

IVF-ET testicular biopsy , TESE/ICSI testicular biopsy 가

Nogueira (1999) Test-yolk buffer 12% glycerol 가

spermatids spermatogenic cell Sertoli cell , 가

spermatogonia, spermatocytes, Sertoli cells, basal tubular compartment , spermatids spermatozoa

spermatogonia, spermatocytes, Sertoli cells, basal tubular compartment spermatids spermatozoa

, 가

, Spermatogonia, spermatocytes, Sertoli cells, basal tubular compartment

spermatids spermatozoa spermatids spermatozoa

mid-piece mitochondria Ben-Yosef (1999)

¹⁴

, - 가 lamina propria folding, Sertoli cell, spermatogonia, spermatocytes round spermatids, elongated spermatids, spermatozoa

, basal compartment

basal compartment

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