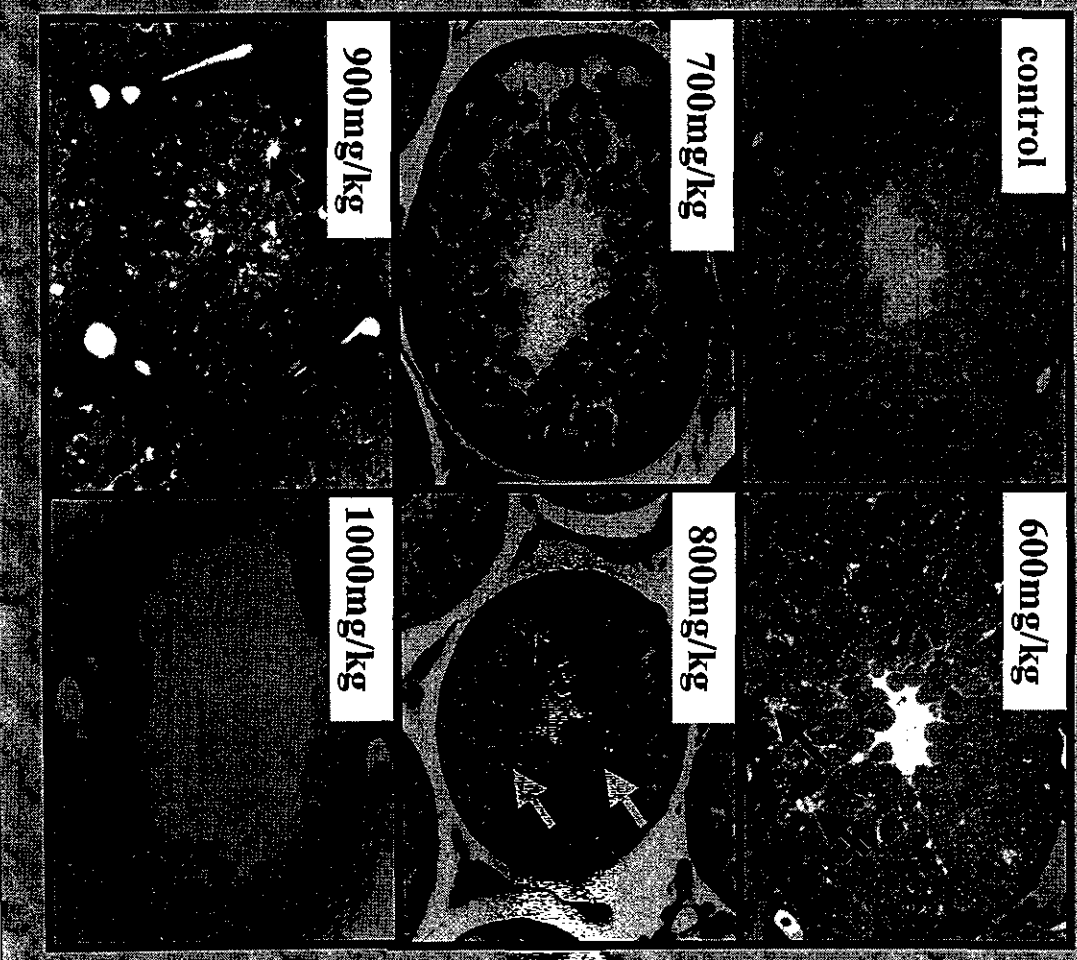


## SEMI-THIN SECTIONS

**Fig. 3-1**

Semi-thin sections of testes of rats fed with various doses of MEHP (Toluidine blue staining) (Mag. x 100).

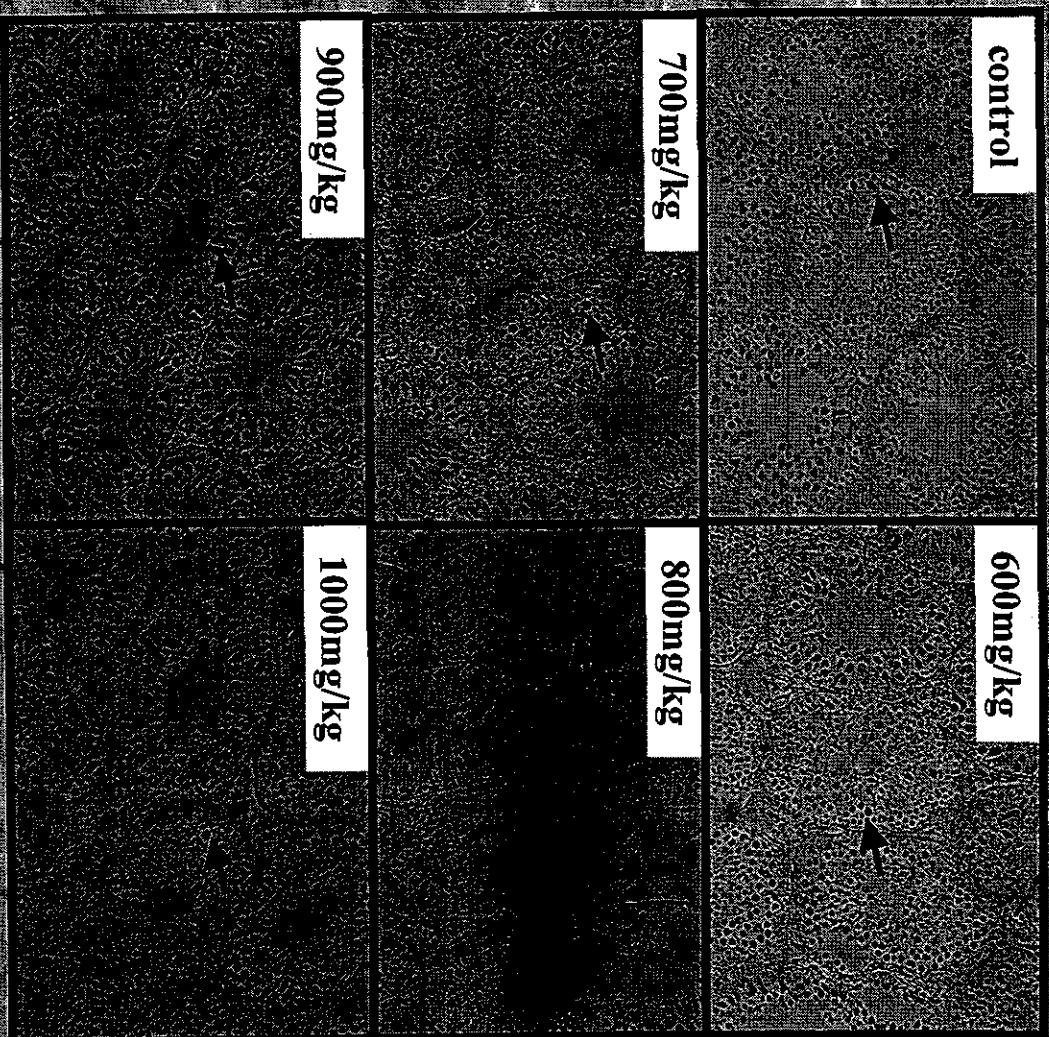
- ↓ spaces between spermatogenic cells;
- ↓ disruption of Sertoli cells;
- ↓ degenerating spermatogenic cells.

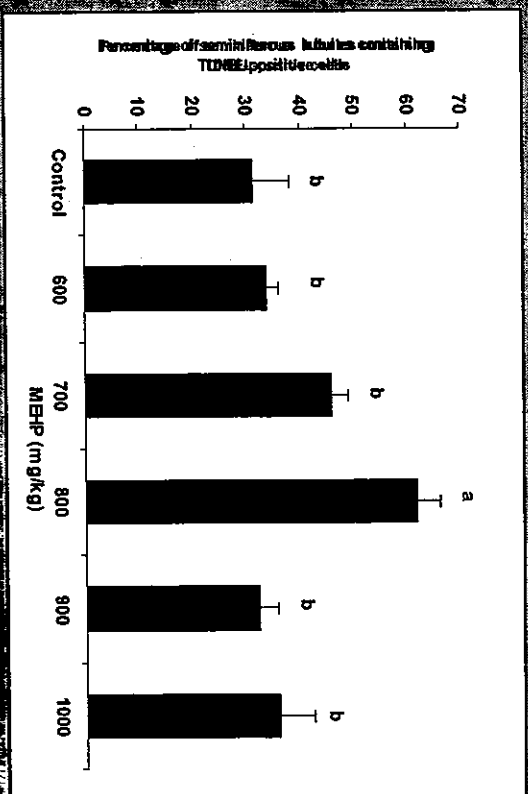
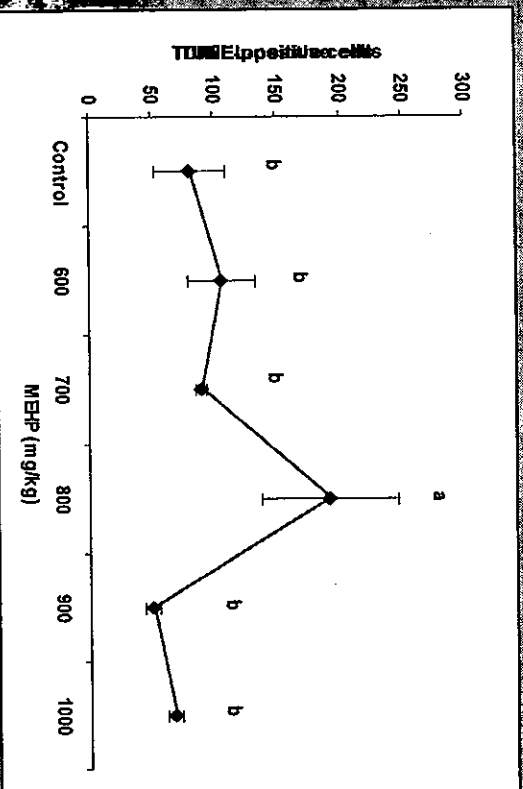


# TUNEL

**Fig. 3-2**

Sections of testes of rats fed with various doses of MEHP (TUNEL apoptosis kit) (Mag. x 50). Arrows indicate TUNEL-positive cells.





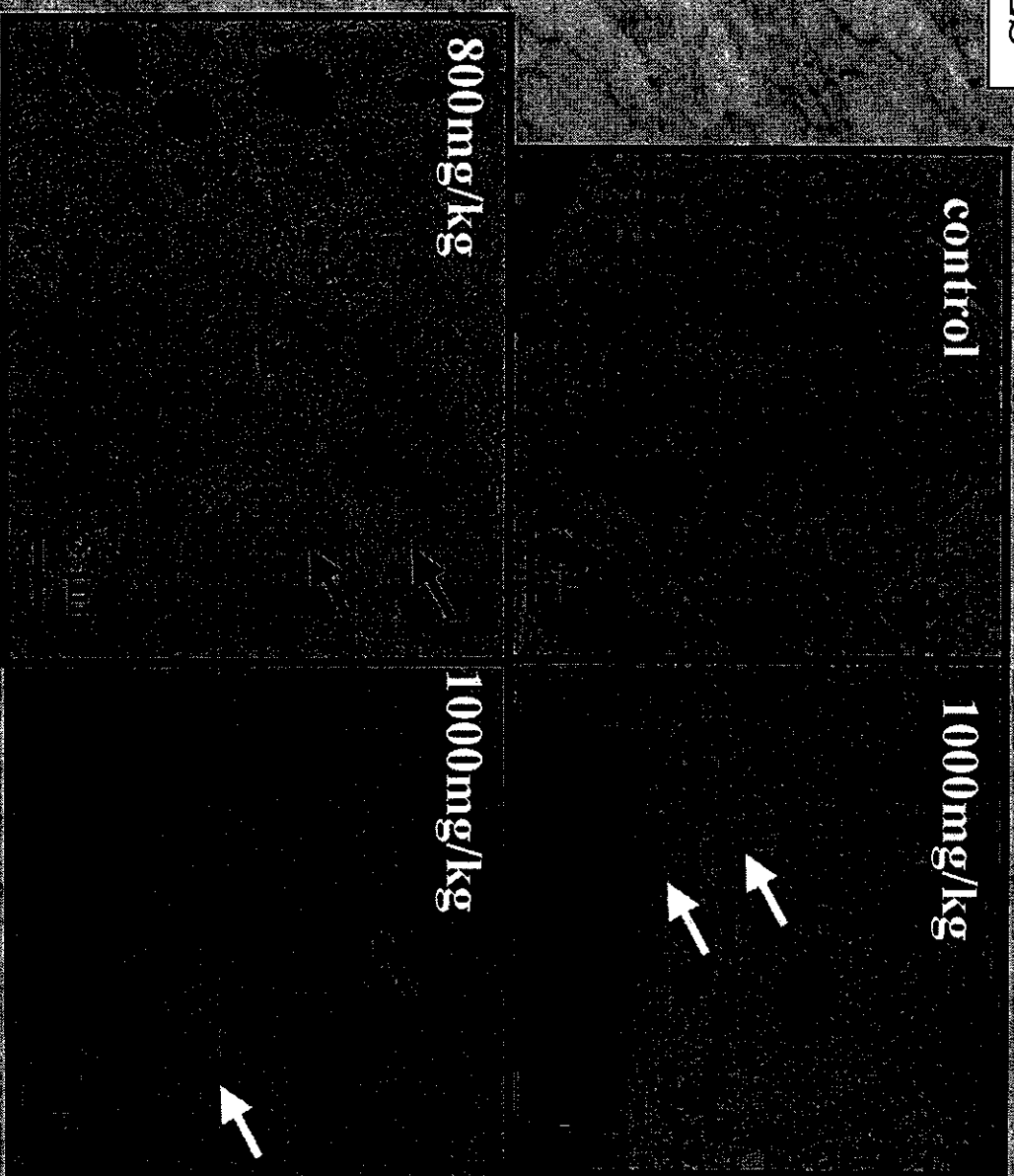
a) Total apoptotic cells in 100 round seminiferous tubules.

b) Percentage of seminiferous tubules with TUNEL-positive cells.

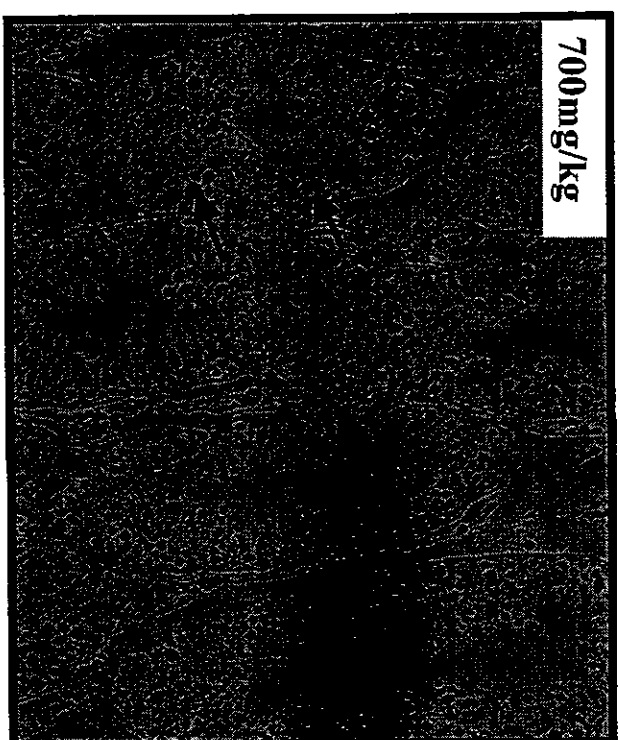
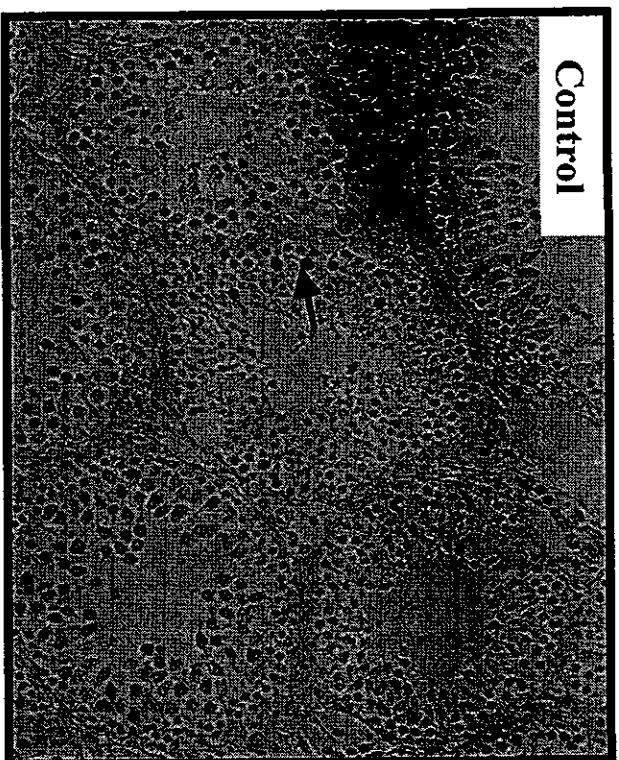
**Fig. 3-3** Total apoptotic cells in 100 round seminiferous tubules and percentage of seminiferous tubules with TUNEL-positive cells in rats treated with various doses of MEHP. Data are presented as mean±SEM and alphabets show Duncan grouping. Rats fed with 800mg/kg MEHP (a) are significantly different ( $p<0.05$ ) from those in rats fed with other doses (b).

## SERTOLI CELLS

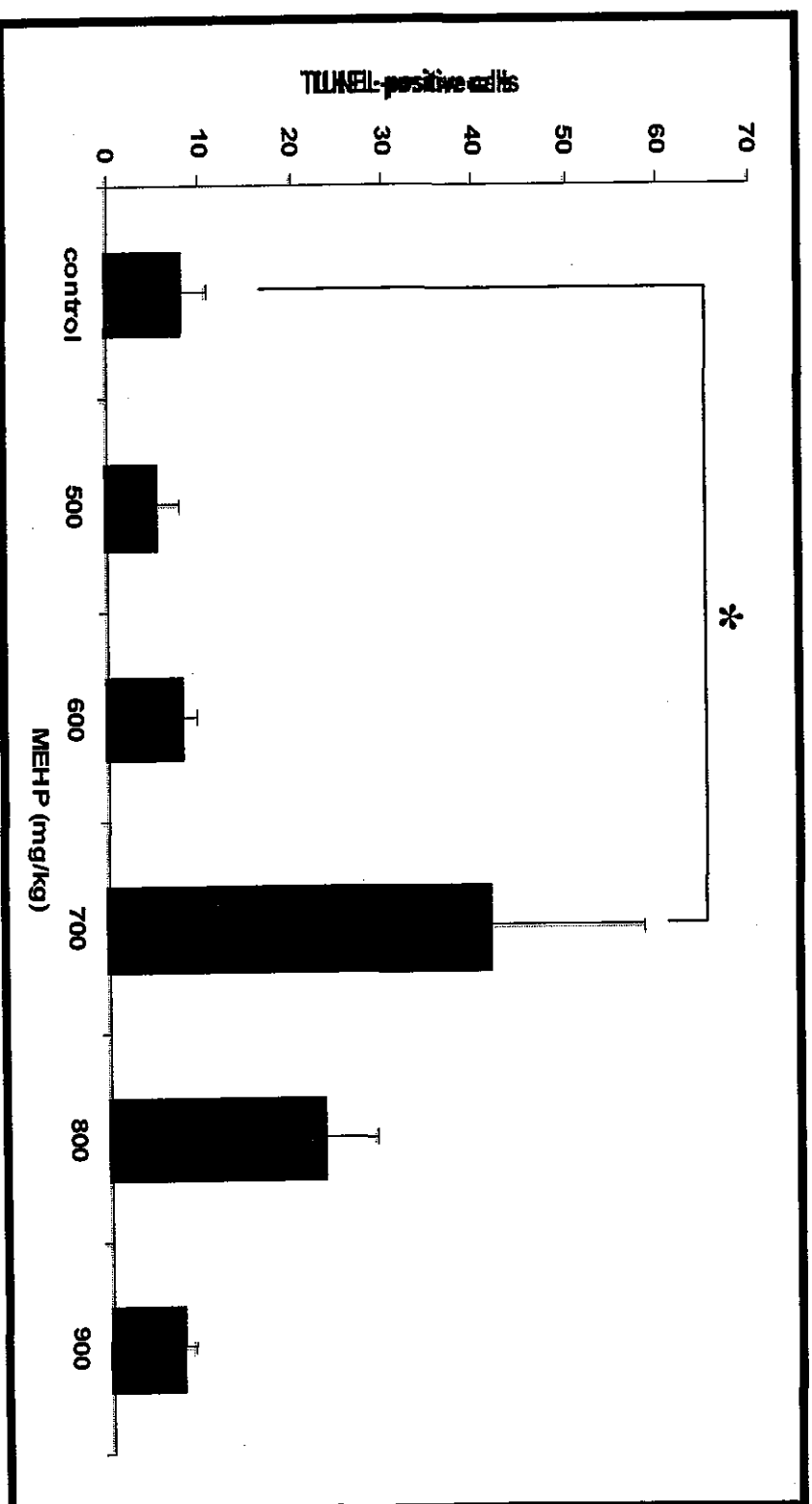
**Fig. 3-4** Sertoli cells in control and treated rats. [top left] Normal Sertoli cell; [top right] Presence of vacuoles (white arrow) in degenerating Sertoli cell; [bottom left] Sertoli cell disruption indicated by large spaces (black arrow); [bottom right] Degenerating Sertoli cell with nuclear membrane lysis (black arrow) and presence of vacuoles (white arrow);



**Fig.3-5 TUNEL**



**Fig. 3-6 TUNEL-positive cells**

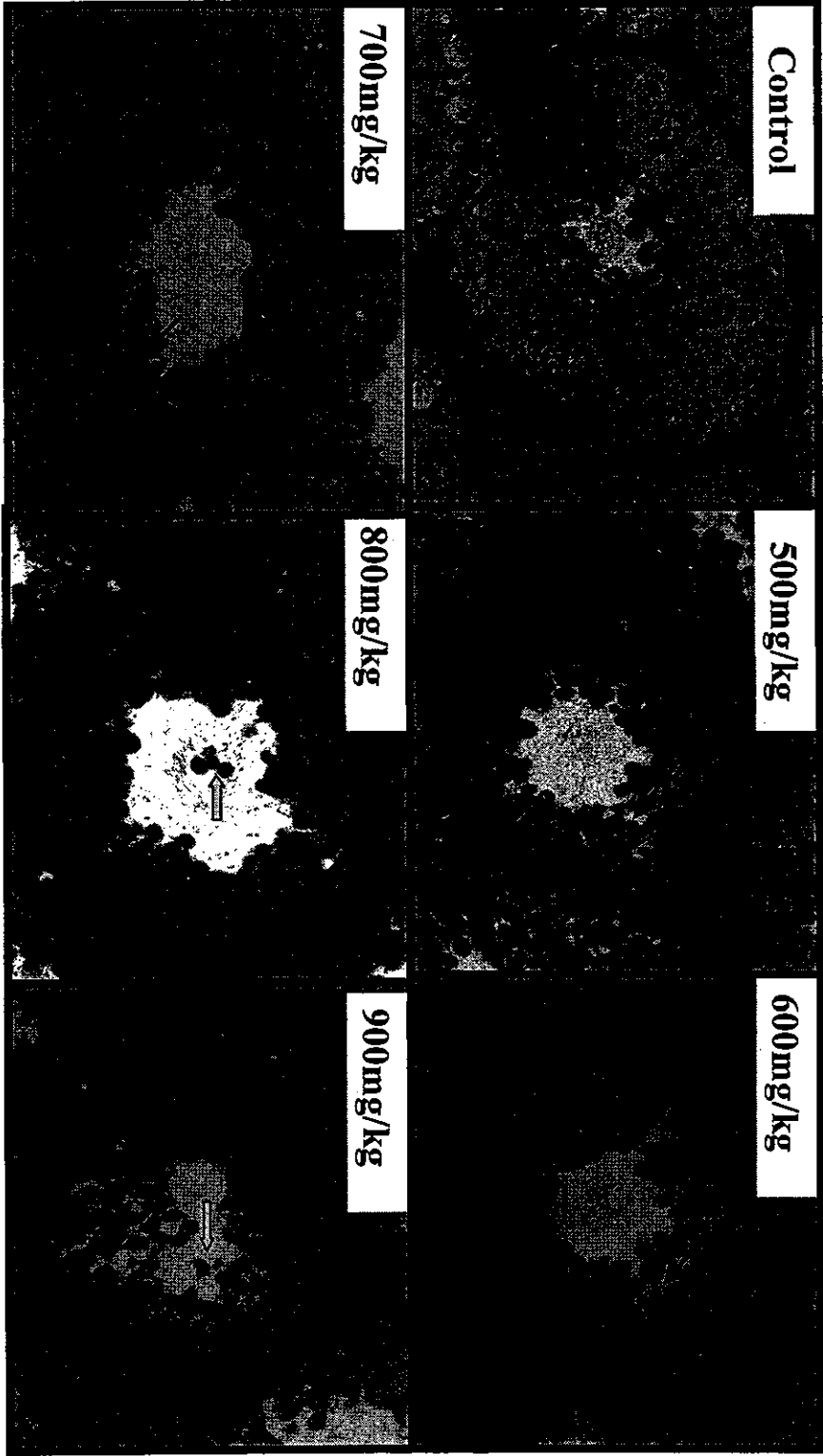


TUNEL-positive cells per 100 seminiferous tubules. (\* =  $p < 0.05$ )



# Fig. 3-7 SEMITHIN SECTIONS

黑矢印：空胞 赤矢印：変性精細胞 黄矢印：脱落精細胞



(Mag. X100)

**Fig. 3-8 TEM**

- a: normal  
spermatogenic cell  
(Mag.x4000)**
- b: degenerating  
spermatogenic cell  
showing margined  
heterochromatin  
(Mag.x4000)**
- c: normal Sertoli cell  
(Mag.x3500)**
- d: degenerating  
Sertoli cell with  
vacuoles (black  
arrow) (Mag.x2500)**

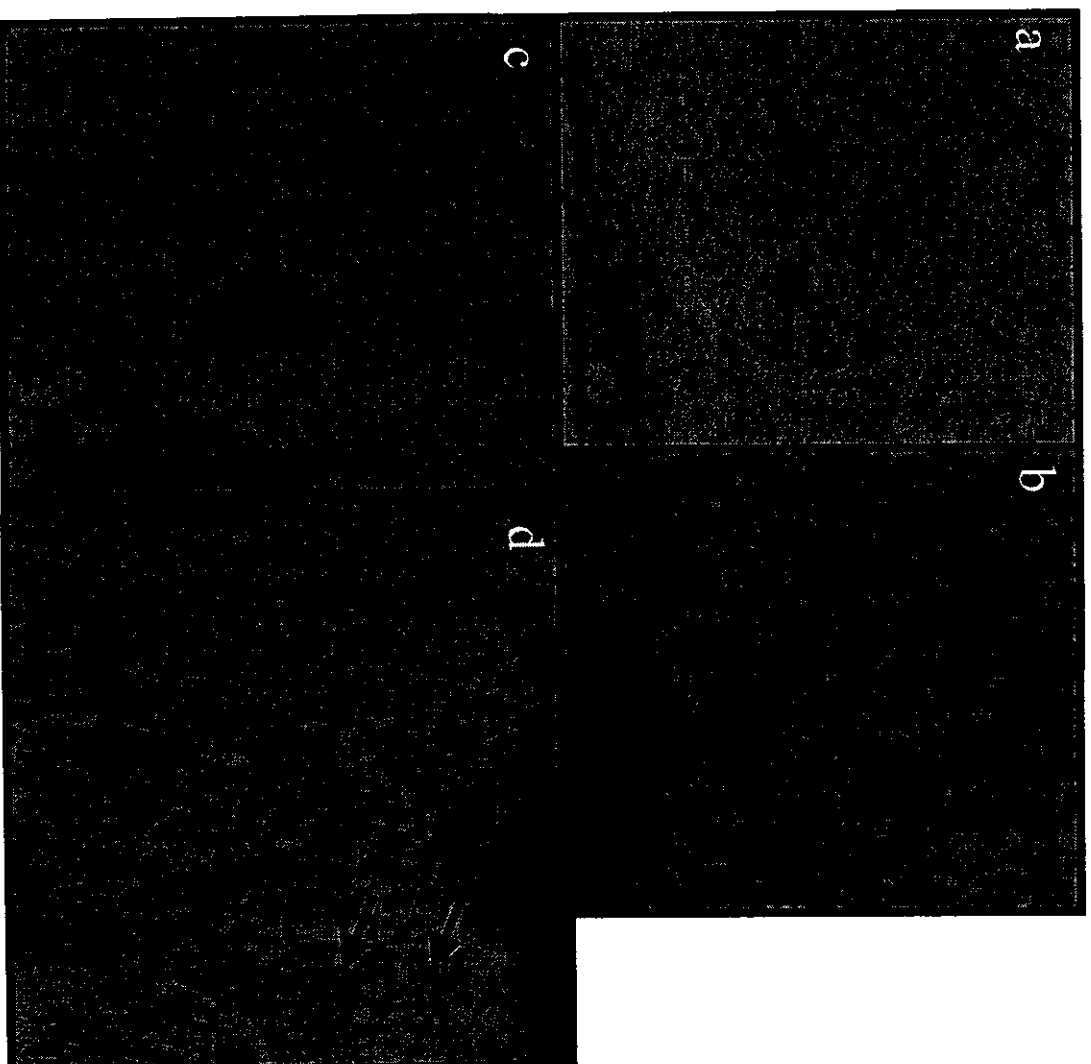
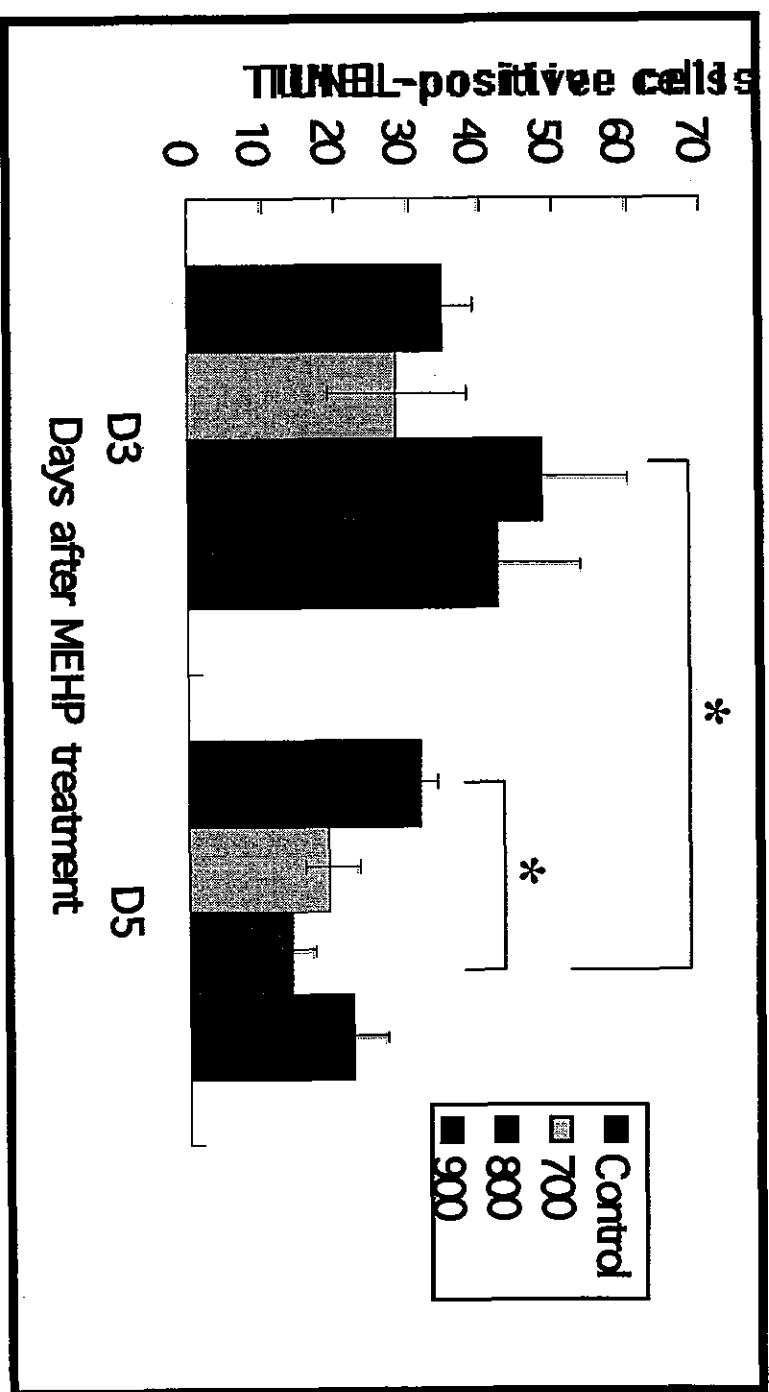




Fig.3-9 TUNEL



TUNEL-positive cells per 100 seminiferous tubules.

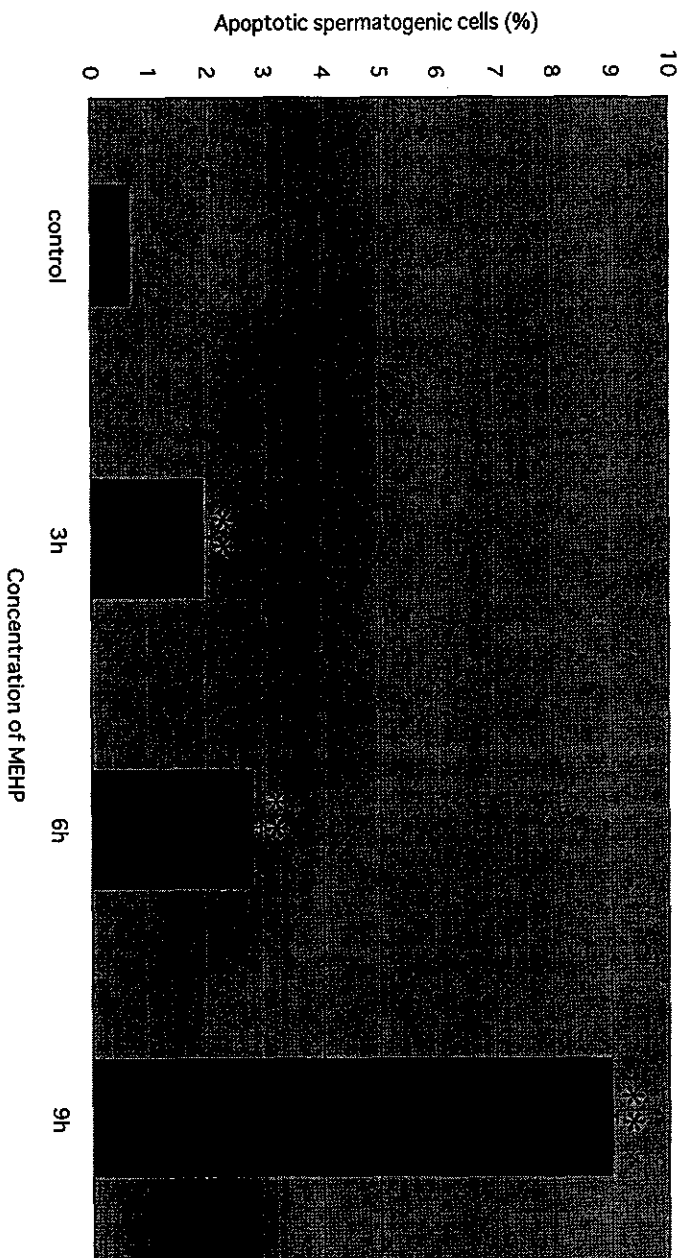


Fig. 3-11 Apoptotic spermatogenic cells increased in a time-dependent manner (\*\* $P<0.01$ )



Fig. 3-12 Necrotic Sertoli cell. The nucleus contains an abnormal vesicle (arrow). Mitochondria distention (\*) and vacuolization (#) are also visible. Treated with  $1 \text{ nmol.ml}^{-1}$  MEHP for 3 hr.



Transmission  
electron micrographs



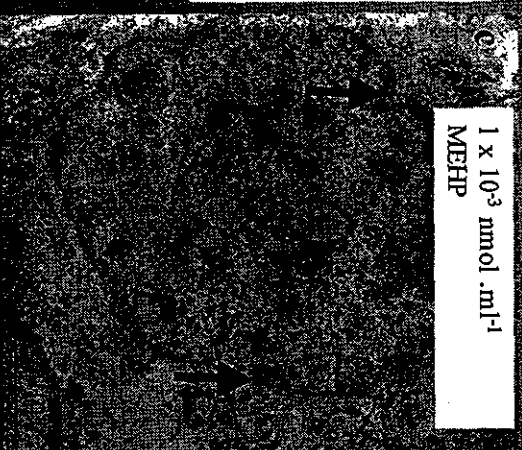
Fig. 3-13 Degenerated spermatogonia at early stage. a) Control. b) Spermatogonia reveal an abnormal appearance (circle). Treated with  $1 \text{ nmol.ml}^{-1}$  MEHP for 3 hr.

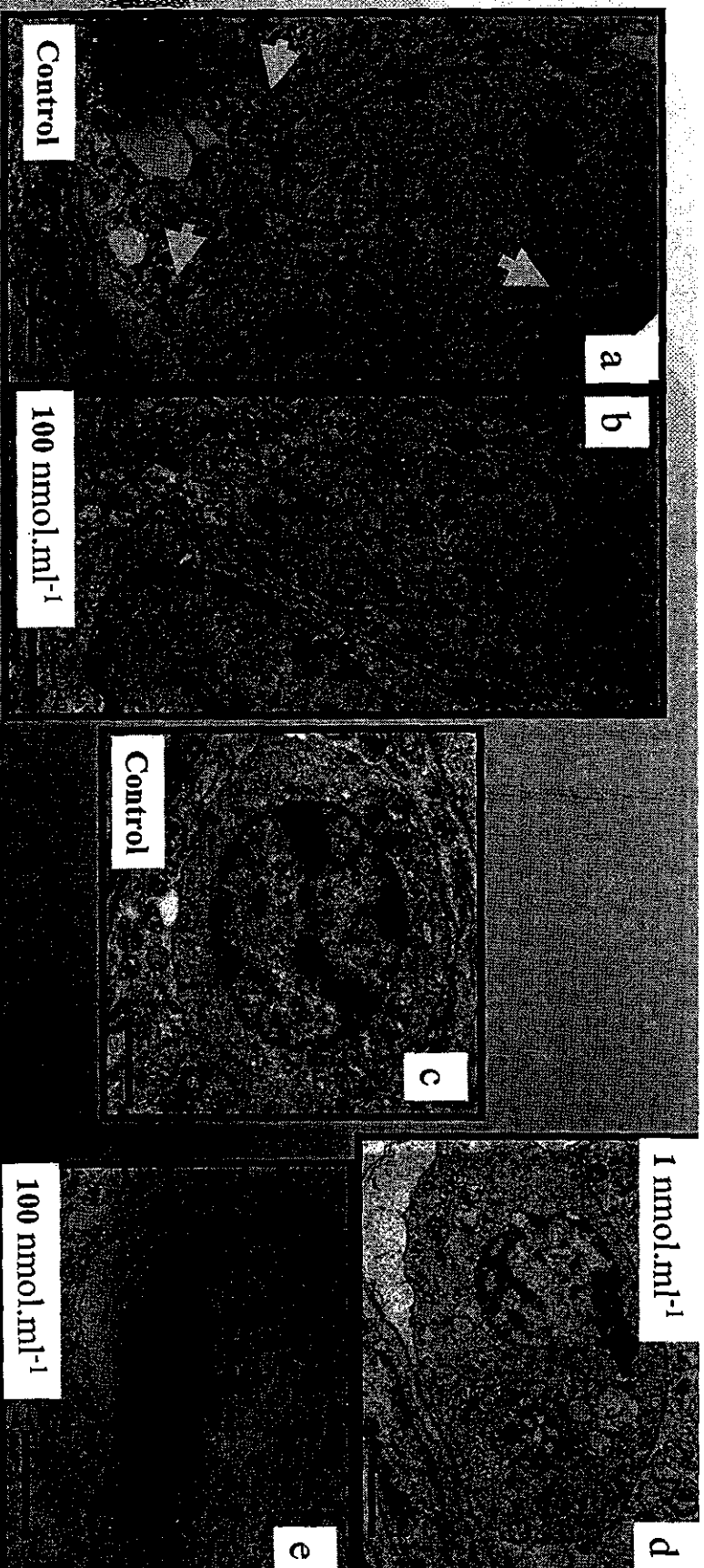
c) Sertoli cell nucleus after MEHP-treatment ( $1 \times 10^{-3} \text{ nmol.ml}^{-1}$ ) for 6 hr. Abnormality of the nucleus can be seen. Some chromatins along the nuclear membrane (arrows) reveal abnormal in appearance.

$1 \times 10^{-3} \text{ nmol .ml}^{-1}$   
MEHP

b) Apoptotic Sertoli cell, control, seeded for 6 hr.

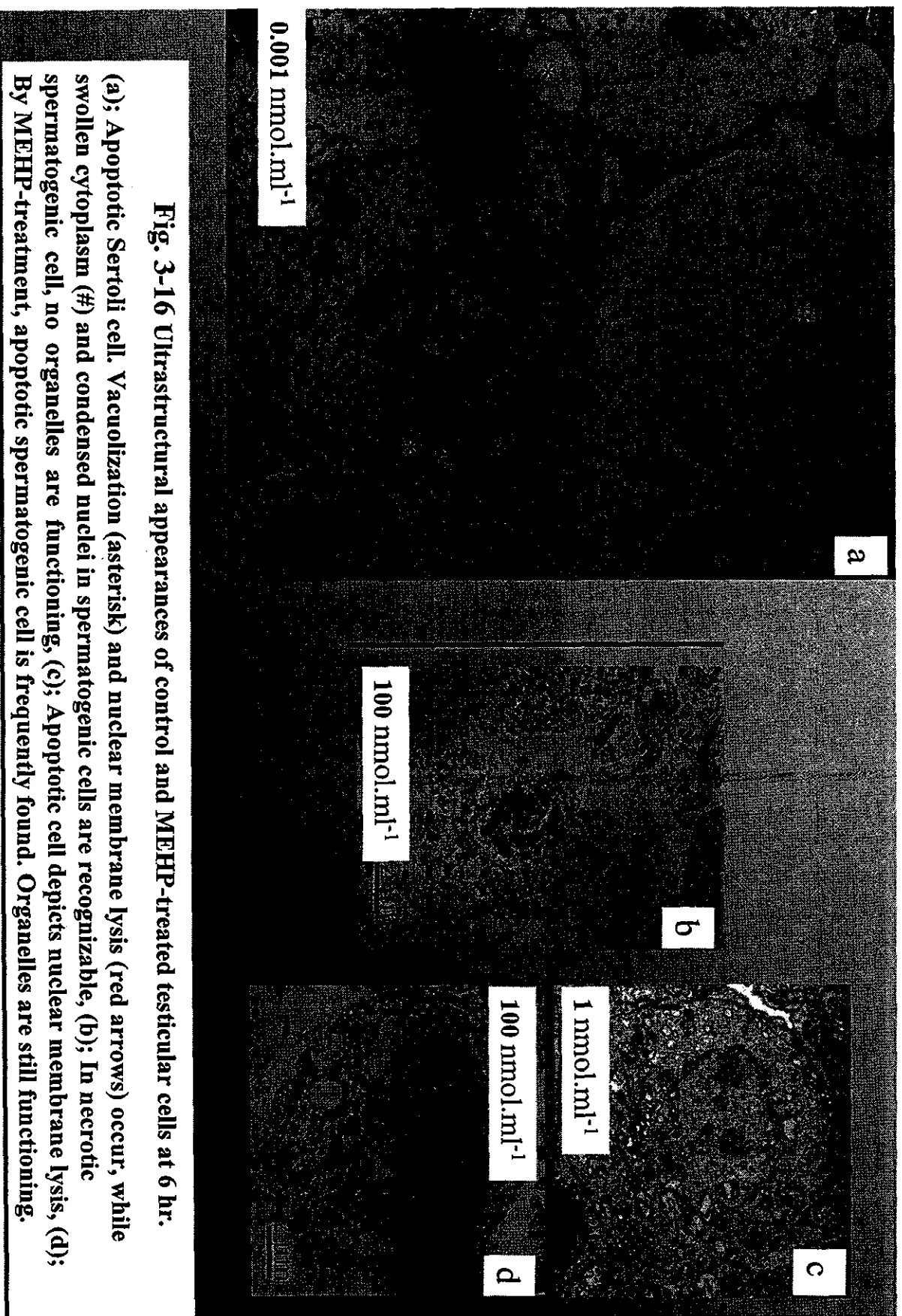
Fig. 3-14 Transmission electron micrographs of testicular tissue culture.  
a) Normal Sertoli cell, control.





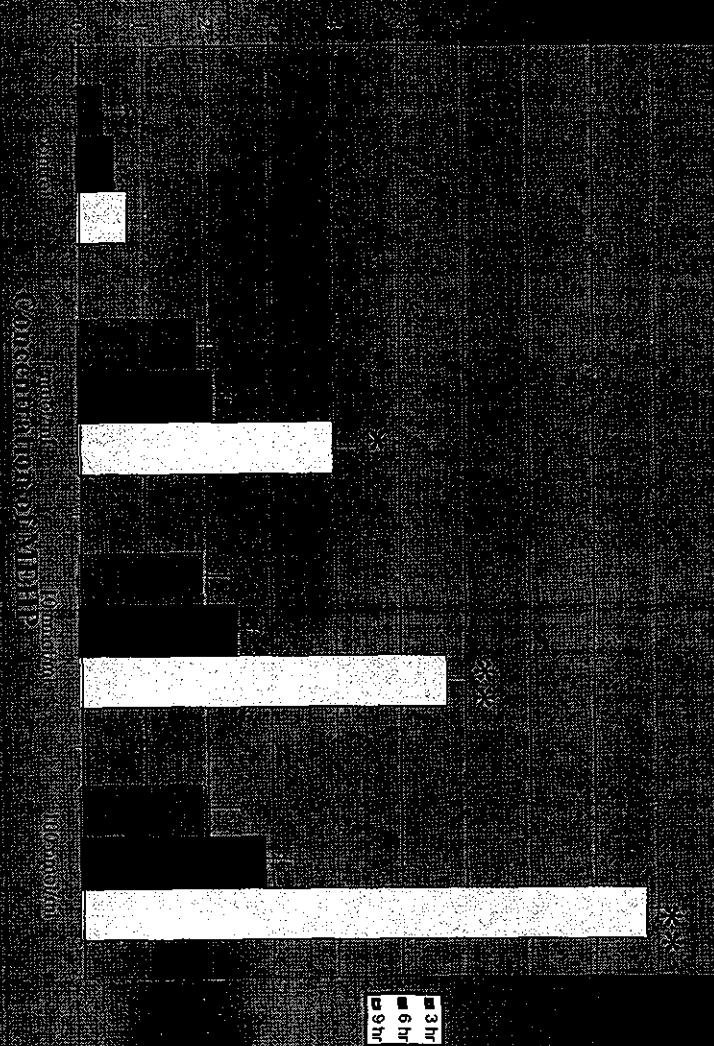
**Fig. 3-15 Ultrastructural appearances of control and MEHP-treated testicular cells at 1 hr after seeding.**

(a); In control Sertoli cell, vacuolization occurs, while mitochondria in normal shape are dominated (yellow arrow), (b); Sertoli nuclear membrane is partly lysed (round marker). It should be the first step of apoptosis, (c); Normal spermatogenic cell in control, (d); Swollen mitochondria (red arrow). It might be the early stage of necrotic spermatogenic cell after administration of 1 nmol/ml MEHP, (e); Apoptotic spermatogenic cell. ScN = Sertoli cell nucleus.



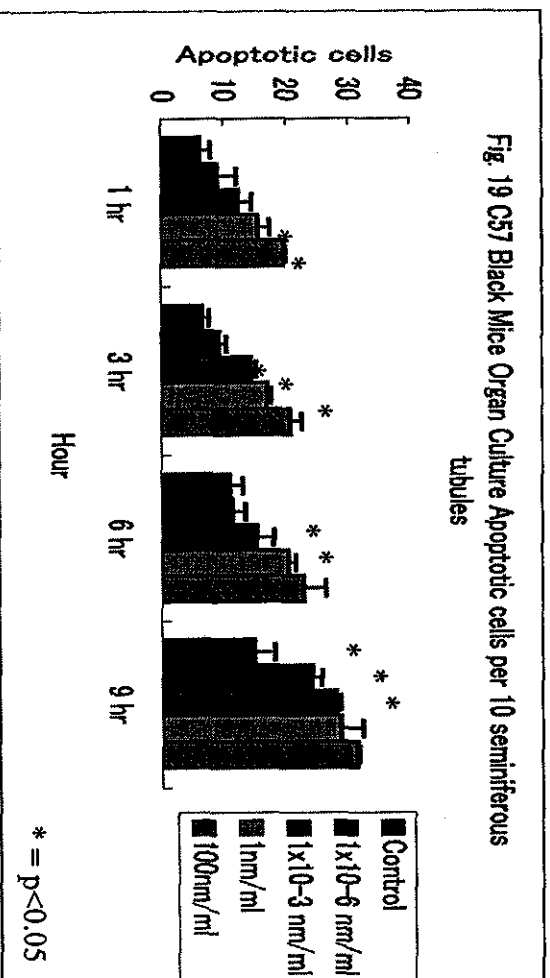
**Fig. 3-16** Ultrastructural appearances of control and MEHP-treated testicular cells at 6 hr.

(a); Apoptotic Sertoli cell. Vacuolization (asterisk) and nuclear membrane lysis (red arrows) occur, while swollen cytoplasm (#) and condensed nuclei in spermatogenic cells are recognizable, (b); In necrotic spermatogenic cell, no organelles are functioning, (c); Apoptotic cell depicts nuclear membrane lysis, (d); By MEHP-treatment, apoptotic spermatogenic cell is frequently found. Organelles are still functioning.

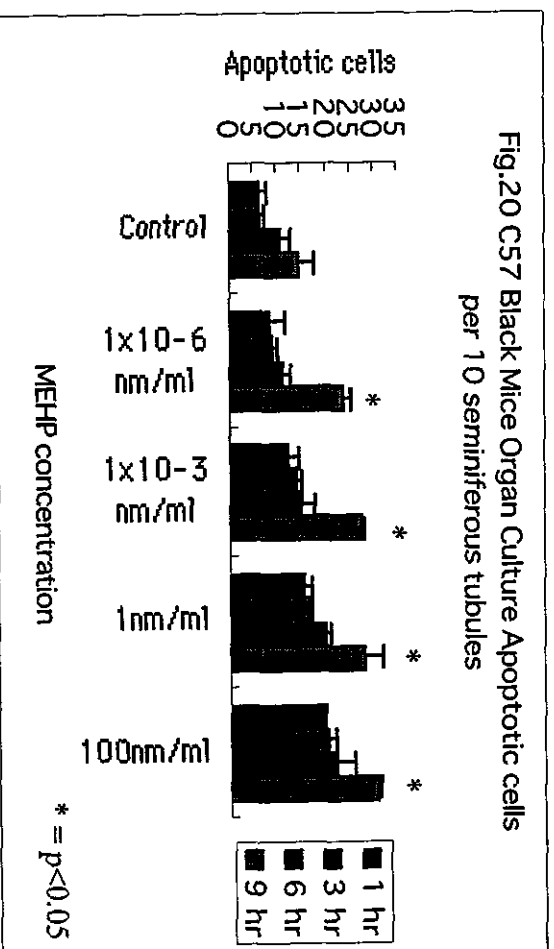




**Fig. 3-19**



**Fig. 3-20**



FasL

Fas

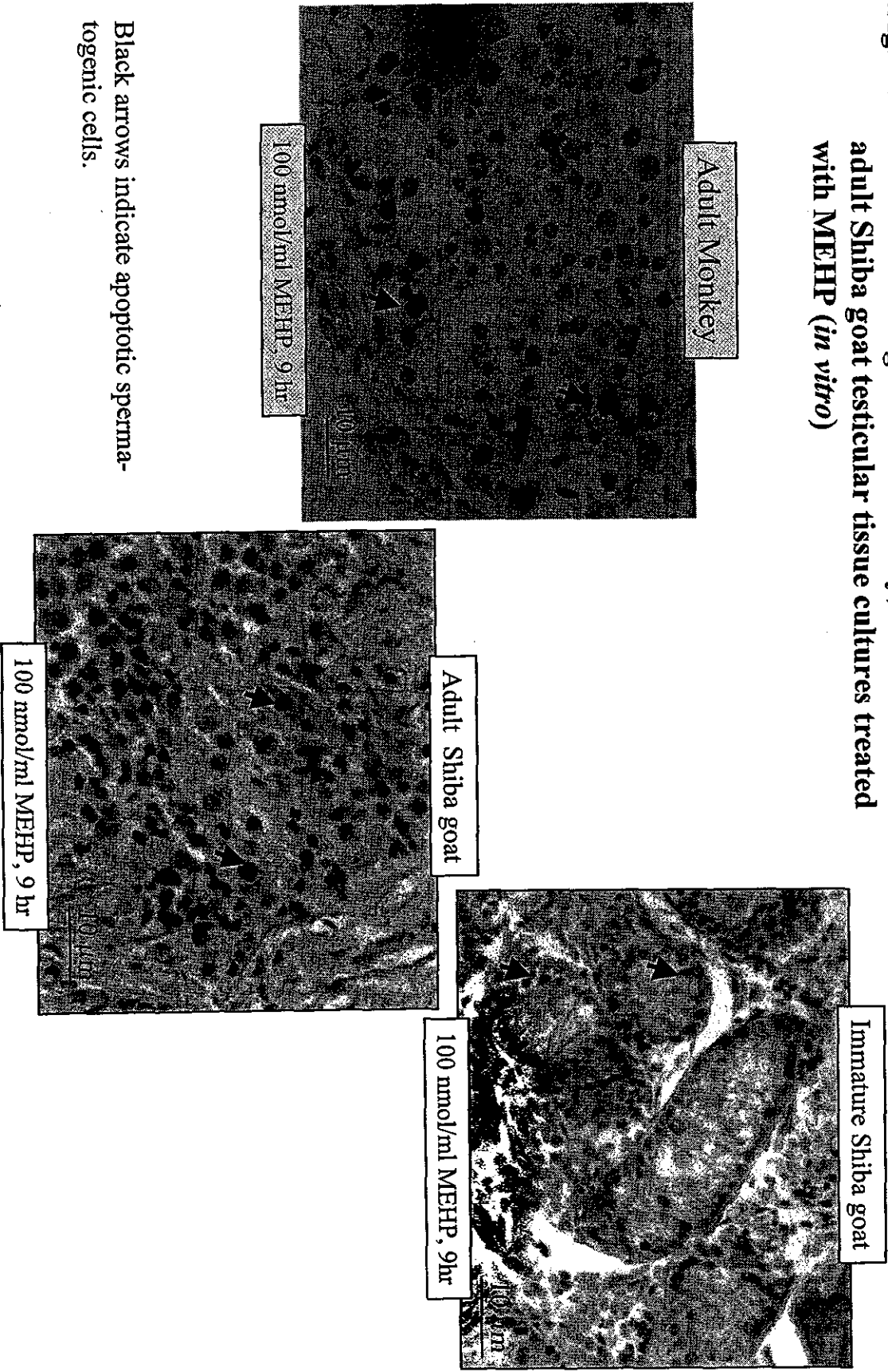
Control 1hr

100 (nmol/ml) 9hr



Fig. 3-21 Black and yellow arrows indicate presence of FasL and Fas, respectively.

**Fig. 3-22 TUNEL staining of adult monkey, and immature and adult Shiba goat testicular tissue cultures treated with MEHP (*in vitro*)**



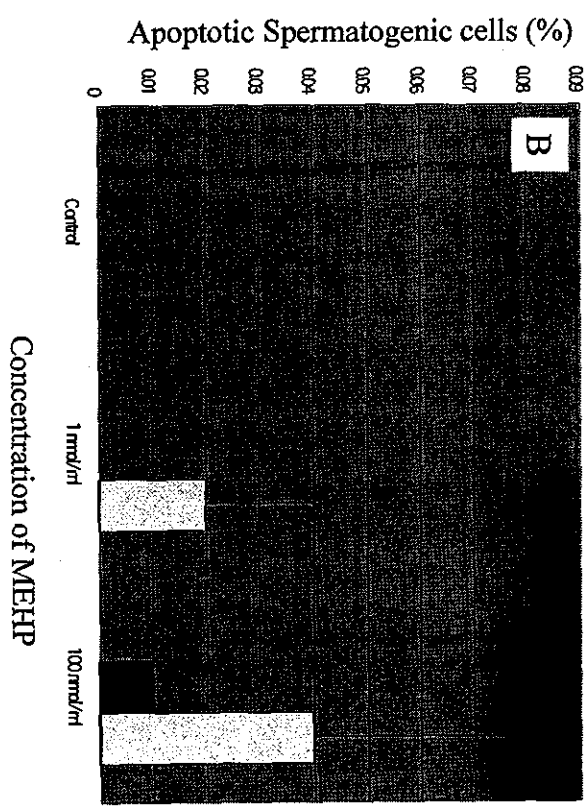
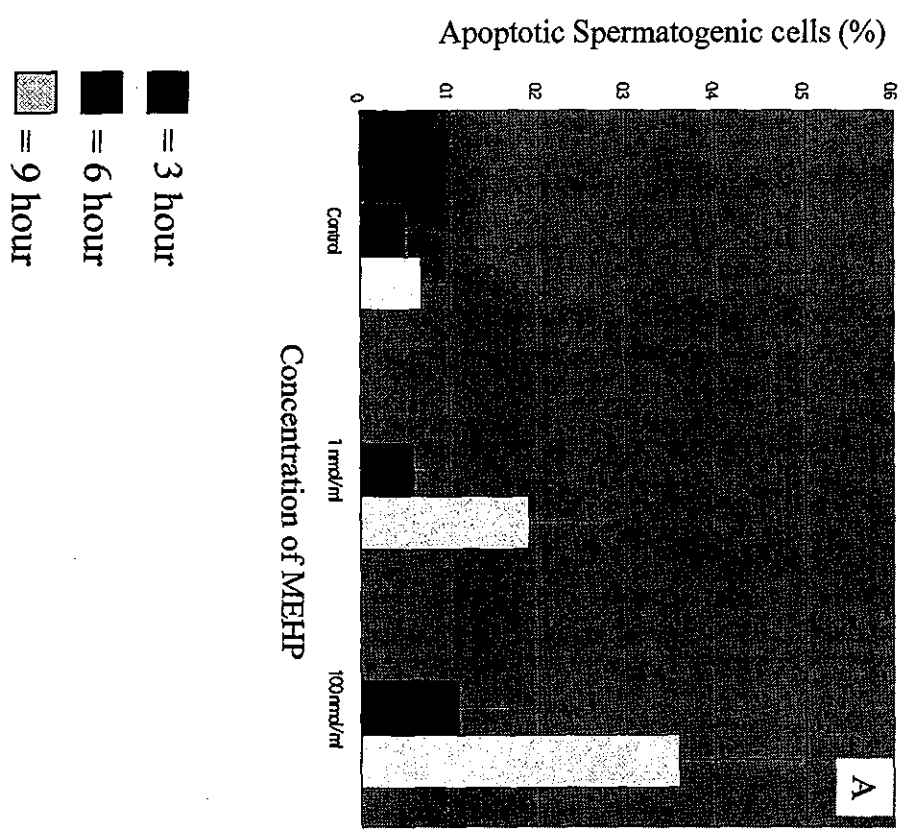


Fig. 3-23 Percentage of apoptotic spermatogenic cells in control and treated groups.

A. MEHP-treatment in the adult monkey testicular tissue culture. Apoptotic spermatogenic cells increase in time-and dose-dependent manners.

B. MEHP-treatment in the adult Shiba goat testicular tissue culture.

Fig. 3-24 Transmission electron micrographs

