Effects of halothane-nitrous oxide exposure on the testicular ultrastructure of mice

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Thirty male Albino mice, ages of which ranged between 10 and 14 weeks, and weights between 40 and 50 g, were selected randomly. The1st and 2nd groups of mice were exposed to halothane-nitrous oxide combination for 5 days, and 10 days, the 3rd group of mice was let to survive 5 more days, and the 4th group for 10 more days in the same environment, after stopping the gas exposure. Testis biopsy materials obtained from al of the groups, were examined by electron microscope (Zeiss EM 10 B).

The first reaction against halothane-nitrous oxide combination in the 1st group was seen in Sertoli cells, which exhibited different electron densities. Some of the Sertoli cells became so dense that they nearly masked cytoplasmic organelles. In the 2nd group, common SER vacuolization, and dense and large phagocytic remnants were visualized in the cytoplasms of these cells. However, in the 3rd group, the presence of centriole near the nucleus was noted in their cytoplasm. In the micrographs of the 2nd group, increment of total thickness of membrana propria, pyknosis of spermatocytes, and acrosomal phase spermatids containing two nuclei with a common and separate acrosome, were observed; what is more, immature and pyknotic spermatids were visualized in the lumen. On the other hand, the Leydig cell cytoplasm revealed completely degenerated mitochondria and increased lipid droplets. In the 4th group, membrana propria, seminiferous tubules and interstitium were seen to exhibit completely normal structures, except some tubules.

After overall evaluation of our results, we concluded that exposure to low halothane-nitrous oxide combination affected Sertoli cells the first, and consequently caused the destruction of spermatogenic cells; however, these degenerative changes were thought to be reversible. [Turk J Med Res 1993; 11(6): 252-260]

Key Words: Halothane, Nitrous-oxide, Testis, Ultrastructure

In epidemiological studies, significant increases in spontaneous abortion and rate of congenital abnormalities of the live-born children were reported among the anesthesiologists, dentists, operating room personnel occupationally exposed to trace amounts of anesthetic gases (1-3).

Various animal experiments were performed to show the toxicity of anesthetic agents, especially inhalation anesthetics. They have different toxic effects on the male and female genital systems in respect to anesthetic dosage and duration of exposure (4-10). Upto date morphological characteristics of testes affected by the external factors were studied extensively and the structural and functional changes were reported (7, 11-17). The testes are affected by the chemical agents too. But there is no study examining the morphologic alterations of testes due to inhalation anesthetice ultrastructurally. So, this study was planned to examine the effect of the halothane and nitrous oxide combinations, which would be probable toxic agents of the atmosphere of operating rooms.

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MATERIALS AND METHODS

10-14 weeks old 30 male Albine mice weighting between 40 and 50 g were selected randomly. In this study we used a closed system having only one entrance and one exit for cases, volume of 360 m^3, temperature of 20 ± 1 °C and 70% humidty. Halothane waas added to the air of the system by the calibrated vaporizes. Nitrous oxide- 1.25 lt/min, 02-1.25 lt/min and Halothane-1 MAC were applied. Eight mice were

Experiment Group	Exposure time to Halothane-Nitrous Oxide (4 hours every day)	Survival time after stopping the Halothane-Nitrous Oxide exposure	Subject Number
1st Group	5 days	-	3
2nd Group	10 days	-	3
3rd Group	10 days	5 days	3
4th Group	10 days	10 days	3

used as control group. Of the other 22,10 mice died and excluded from the experiment. The remaining 12 Albino mice were held in a closed system containing Halothane-nitrous oxide combination 5 days, 4 hours every day. At the and of 5th day the testes of 3 mice were excised (1st Group). The romaining 9 mice were held in the system for another 5 days. At the end of this period the testes of 3 mice were excised (2nd Group). The remaining 6 mice were held in the same environment for another 5 days, but halothane-nitrous oxide combination was not given. At the end, the testes of 3 mice were excised (3rd Group). The last 3 mice were lax to survive 5 days more (a total of 20 days) and then teheir testes were excised (4th Group).

The control group was held in a similar closed system containing only room air. At the end of each periods the testes of 2 mice from the control group were excised. The animals were let to feed and drink between the experiment periods.

After each period the animals were held in the room temperature for 30 minutes. They gained all of their activities 10 minutes after stopping exposure without no need to stimulants.

Tissue pieces for electron microscopy were fixed in the Glutaraldehyde solution (pH-7.4) prepared with Milloning phosphate buffer at +4°C for 4 hours. Later on 2nd fixation was performed in the Osmium tetroxide (Os02) solution and they were dhydrated by using ethyl alcohol. Minute tissue pieces were embedded in araldite and polymerized at 60 °C for 48 hours. Five hundred A° thick sections were cut by Reichert OMU 3 ultramicrotome and stained with uranyl acetate and lead citrate solutions. Stained sections were examined under ZEISS EM 10 B electron microscope.

RESULTS

In the testes biopsy materials of the 1st and 2nd groups exposed to halothane nitrous oxide combination and of the 3rd and 4th groups existing after stopping exposure membrana propria, seminiferous tubules and interstitium were examined by electron microscope.

Although significant degenerative changes were not revealed in the layers of membrana propria in the 1st group, irregular and bilaminar basal lamina in some tubules and the small invaginations of the basal lamina towards the tubule were observed. Besides the insigrificant increase in the collagen fibrils in the internal non-cellular layer, irregularities in the myoid cells were also seen (Figure 1). In some sections membrana propria was almost normal in structure.

Different electron densities were observed in Sertoli cells in halothane-nitrous oxide combination. Although some of them were showing low electron density the others were so electron dense masking the cytoplasmic organelles (Figure 1a). The nucleus was irregular and lost its typical deep invaginations in most of the cells. There were minimally dilated SER cisternae, a few lipid droplets and dense mitochondria with wide tubular cristae in the cytoplasm. Some Sertoli cells seemed foamy because of the excess dilatation of SER cisternae (Figure 1).

Cytoplasmic bridges were noted between the spermatogonia based on the basal lamina. These cytoplasmic bridges appeared between all of the spermatogenic cells. In these cells the nucleus had regular borders and there were a few vacuoles and minimally degenerative mitochondria in the cytoplasm (Figure 1). In the micrographs some spermatocytes had nearly normal structure, but some were pyknotic. Grouped mitochondria, membranous structures and developed Golgi complex were present in the cytoplasm of the spermatocytes. The acrosomal degeneration was apparent in the spermatids. Acrosomal vesicle was irregular and wavy in these cells (Figure 2).

The nucleus, had irregular borders in Leydig cells and the chromatine appeared as dispersed foci benezath the nuclear membrane. Increased amount of lipid droplets, degenerative mitochondria and vacuoles were frequently observed in the cytoplasm (Figure 3).

Degeneration was more apparent in the 2 group micrographs. Total thickness of the membrana propria was increased in comparison to the 1st group. Basal lamina became diffusely bilaminar and irregular. Collagen fibrils increased significantly in the internal non-cellular layer. Myoid cells were highly electron dense and irregular (Figure 4).

Some Sertoli cells appeared normal in electron density while the others had minimal electron density. Some of their nuclei had irregular border, deep invaginations and the others had oval structure (Figure 4,5).

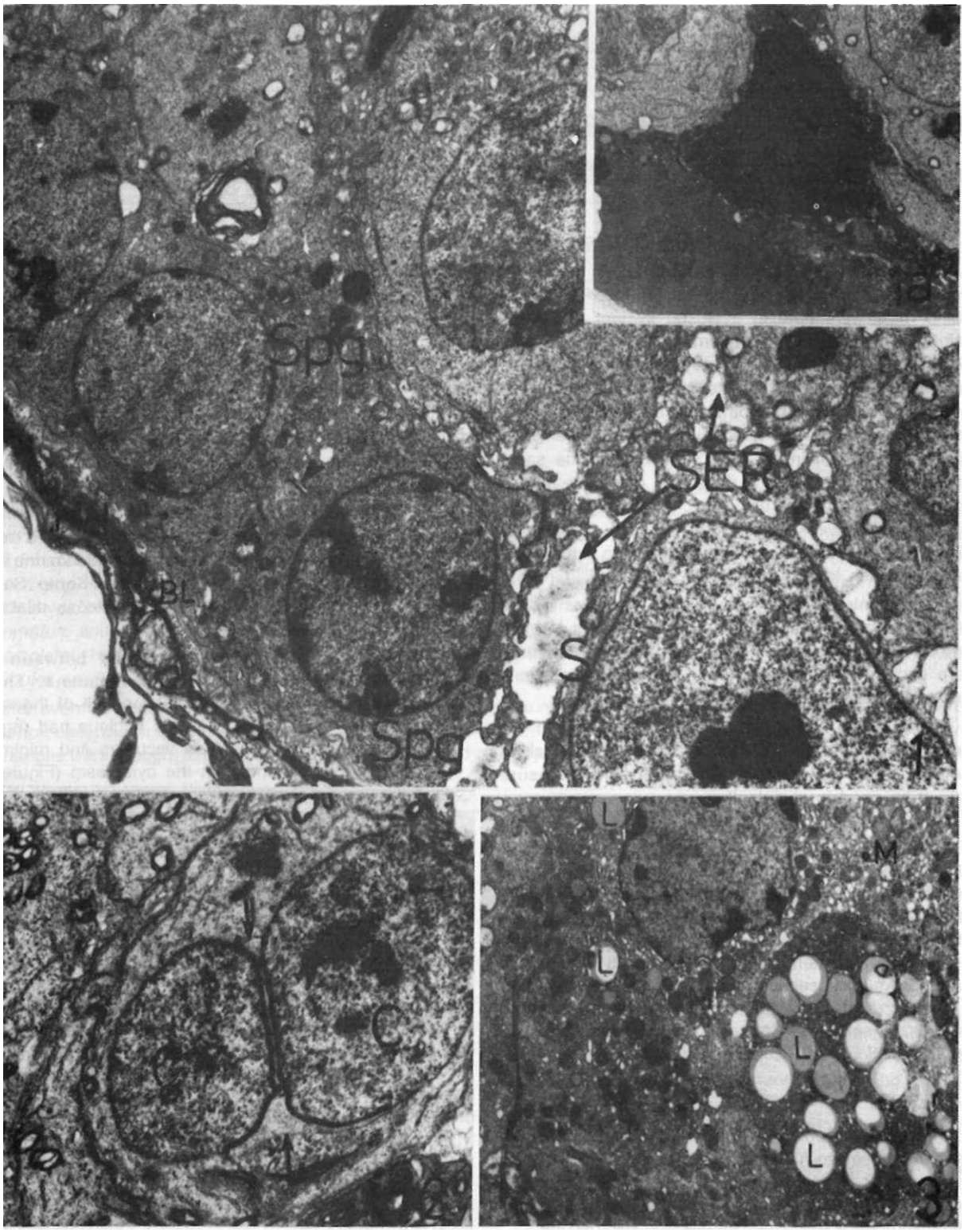

Figure 1. Bilaminar basal lamina (BL), irregular myoid cells (MC) in membrana propria. Spermatogonium (Spg) bound by cytoplesmic bridge (arrow). SER vacuolization (arrows) in Sertoli cell (S). 1st group, x 6.300. Figure 1a. Electron dense Sertoli cell. 1st group. 4.725. Figure 2. Abnormal spermatid with two nuclei (N) and shared acrosoma (arrow). 1st group. x7.440. Figure 3. Increased lipid droplets (L) in Leydig cell cytoplasm. Mitochondria (M). 1st group, x 4.860.

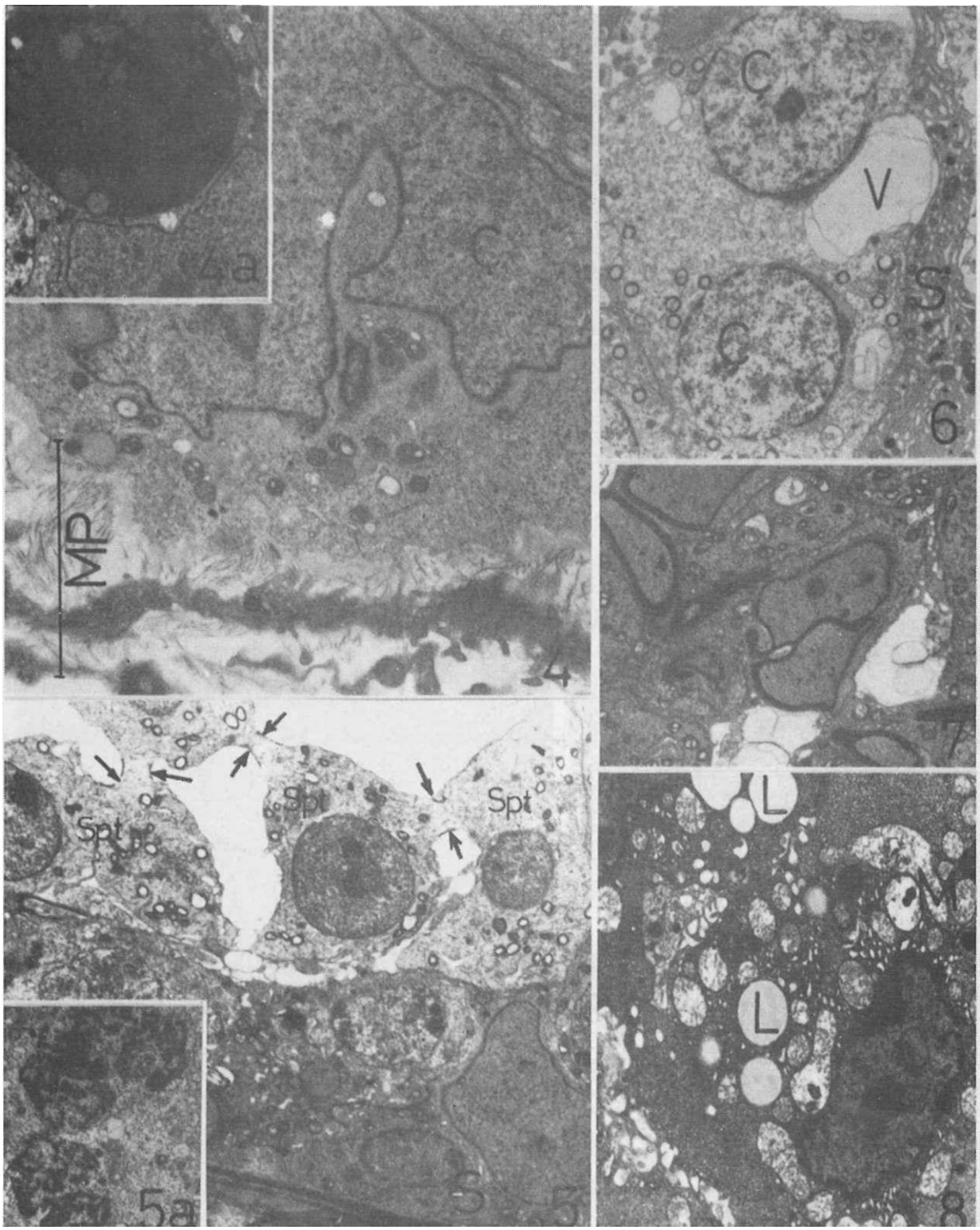

Figure 4. Membrana propria (MP) with increased total thicknees. Sertoli cell nucleus (N) with deep invaginations. 2 nd group.x8. 100. Figure 4a. Dense phagocytic remnants in Sertoli cell cytoplasm. 2nd group.x3. 700. Figure 5. Narrowing in tubular epithalium. Spermatocytes (Spt) bound by cytoplasmic bridges (arrows). Sertoli cell (S). 2nd group. x3. 780. Figure 5a. Spermatocytes with pyknotic nuclei. 2nd group, x 3.000. Figure 6. Spermatid having two separate acrosomes and two nuclei (N) Vacuolo (V), Sertoli cell (S). 2nd group, x 3.780. Figure 7. Abnormal acrosomal phase spermatids. 2nd group. x3.780. Figure 8. Completely degenerated mitochondria (M) Leydig cell with irregularly outlined nuclous (N). Lipid droplets (L). 2nd group.X7.440.

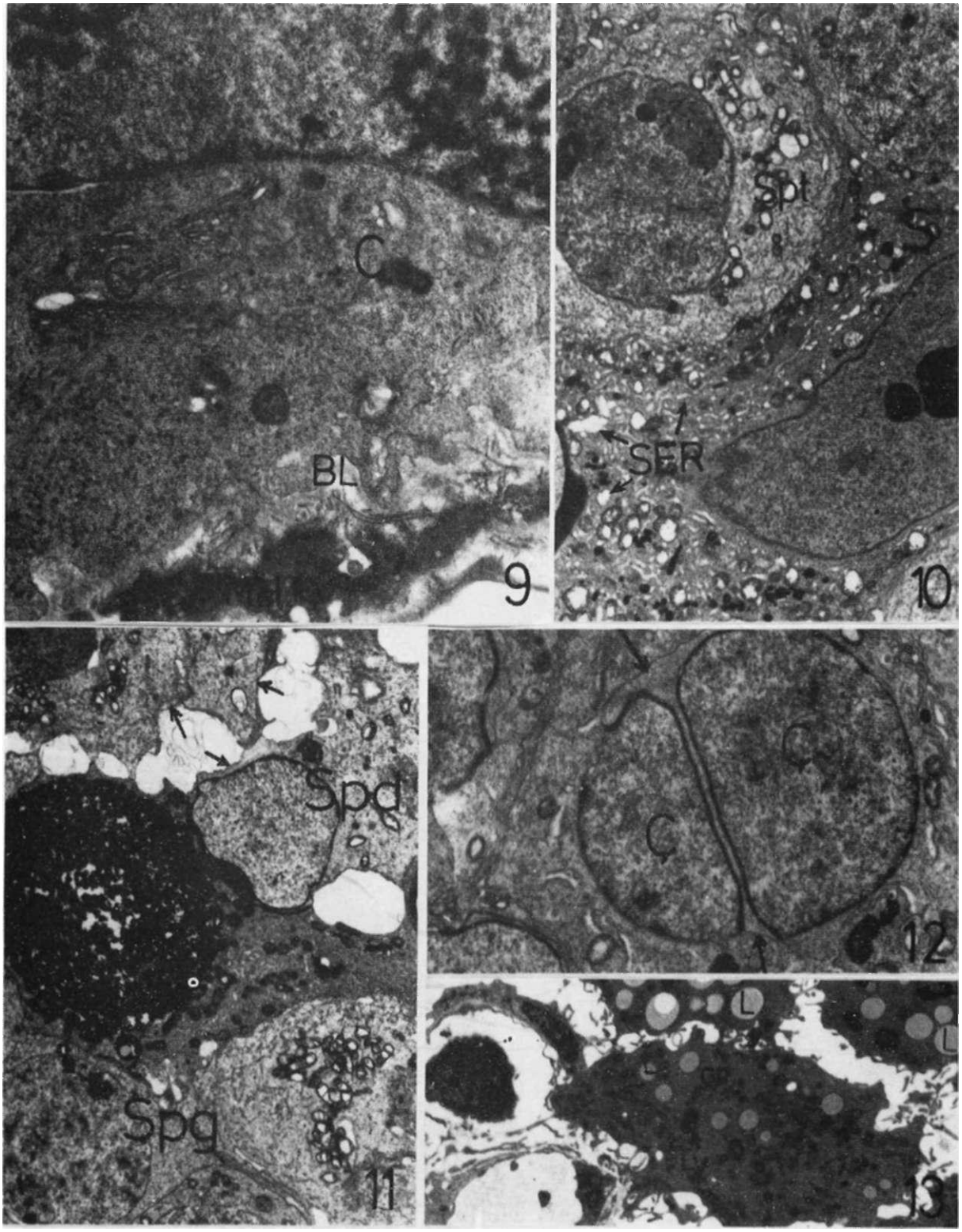

Figure 9. Irregular basal lamina (BL) In membrana propria, vesicular and dense myoid cell (MC). Centricle (C) in Sertolli cell cytoplasm, multiple golgi complexes (G). 3 ^{d} group,x9.660. Figure 10. SER vacuolization (arrows) in Sertoli cell (S) cytoplasm. Dense and grouped mitochondria spermatocytes (Spt). 3 ^{d} group, x 4.860. Figure 11. Irregular spaces between spermatogenic cells (arrow). Spermatogonium (Spg), spermatid (Spd). 3 ^{d} group. x3.780. Figure 12. Abnormal spermatid with two nuclei (N) and shared acrosome (arrows). 3 ^{d} group, x 7. 440. Figure 13. Lipid droplets (L) and lysosomal structures (Ly) in Leydig cell cytoplasm. 3 ^{d}. group x 3.780.

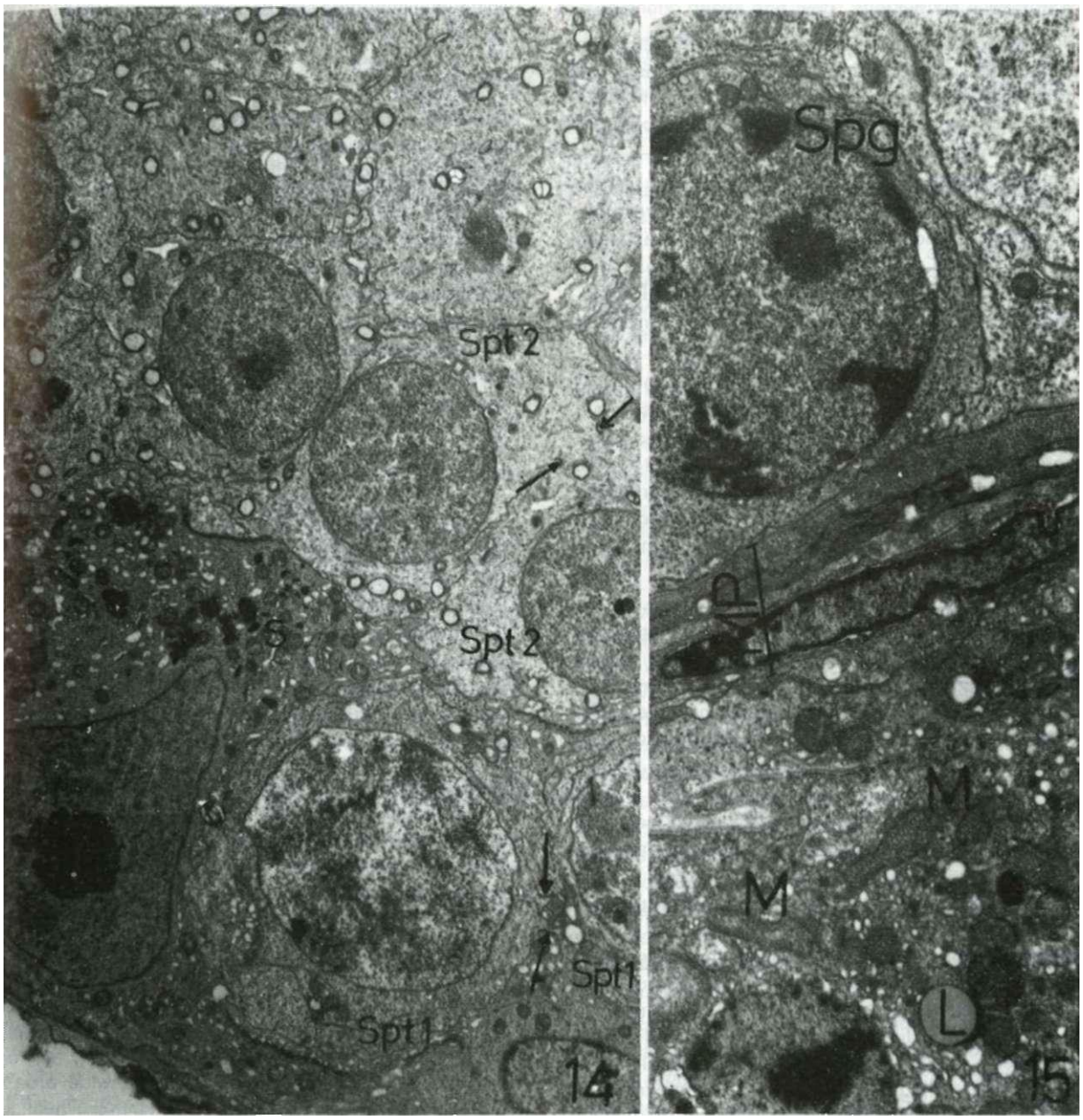

Figure 14. ICytoplasmic bridges (arrows) between primary spermatocytes (Spt 1) and between secondary spermatocytes (Spt 2). Sertoli cell (S) with normal density. 4th group, x 5. 000. Figure 15. Normal membrana propria (MP), spermatogonium (Spg) a few lipid droplets (L) in Leydig cell cytoplasm, tubular mitochondria (M). 4th group. x10.000

These cells were contained swollen and dense mitochondria, lipid droplets, a few lysosomal structures and phagocytic remnants Phagocytic remnants were composed of lipid droplets, vacuoles, mitochondria and dense structures (Figure 4 a).

Spermatogenic cells were more degenerative. Wide irregular space were around the shrunk spermatogonia. Some spermatocytes were shrunk due to degeneration (Figure 5) where as the nuclei of spermatocytes in some tubules were pyknotic (Figure 5 a). Spermatids having abnormal acrosomal membrane, two nuclei and shared acrosomes were also noted (Figure 7) all together the spermatids with two sparets acroso-mes and two nuclei (Figure 6). Large vacuoles and dense mitochondria were seen in the cytoplasm of these abnormal spermatids. The most typical finding in these micrographs was the narrowing in the tubular

epithelium due to the decreased number of spermatogenic cells (Figure 5).

The nucleus had irregular, wavy structure in Leyding cells with intermediate electron density. In their cytoplasm there were lipid droplets, completely destroyed mitochondria and widened SER tubules in some areas (Figure 8).

The findings of membrana propria in the micrographs obtained from the 3rd group were almost similar to those of the 1st group. It was seen that basal lamina was bilaminar in some areas and minimally irregular, collagen fibril increased insignificantly and myoid cells were dense and irregular (Figure 9).

Besides Sertoli cells with irregulary outlined nuclei, swollen mitochondira, widened SER cisternae, a few lipid droplets, secondary lysosomes and, numerous electron dense structures (Figure 10), the cells with normal density and structure were also seen. In these cells centrioles were observed close to the nuclei in addition to multiple Golgi complexes, mitochondria, polysomes, SER and RER (Figure 8).

Although spermatogonia had normal structure the spaces in different dimensions around some shrank spermatogenic cells were observed (Figure 11). Acrosome interrupted over the nucleus in some areas in acrosomal phase spermatids. The spermatids with shared acrosome and two nuclei were seen in this group also (Figure 12).

The cytoplasms of Leydig cell located near the capillaries were include lysosomal structures, multiple Golgi complexes and lipid droplets (Figure 13).

It was seen that some seminiferous tubules gained almost the normal structure in the 4th group. Membrana propria was normal. Basal lamina seemed regular and homogeneous (Figure 14,15). Sertoli cells were also normal except in soma tubules. The nucleus was in typical structure with invaginations and had 1 or 2 nucleoli. However, SER dilatations, apical dence structures, rare lipid droplets, Golgi complexes and mitochondria some of which had degeneration were seen in Sertoli cell cytoplasm in some tubules (Figure 15). The junctions between Sertoli cells and between spermatogenic cells were as normal as all the other groups.

Spermatogania had normal electron density and lined regularly on the basal lamina (Figure 15). Cytoplasmic bridges were seen between the primary and secondary spermatocytes during division (Figure 14). Acrosomal phase spermatids with developed Golgi complex, scattered mitochondria and nuclei with regular border were normal except some pyknotic spermatids between the tubular epithelial cells.

There were a few lipid droplets, Golgi complexes, minimally dilated SER tubulec and tubular mitochondria in Leyding cells located in the interstitium (Figure 15).

DISCUSSION

Halothane used commonly by anesthesiologists as inhalation anesthetic is a strong anesthetic with a low solubility in blood and high potential for rapid anesthesia induction. Nitrous oxide is a weak analgesic and anesthetic agent and used with another potent anesthetic and oxygen. In the past, nitrous oxide was shown to be cytotoxic in the plant and animal cells so it was thought to be used especially in the treatment of leukemias, Later on, it was used in the studies related to the reproductive system since it affected DNA synthesis, caused inhibition of the mitotic spindle assembly with a cofchicine-like activity and disturbed the lining of chromosomes (2). It was reported that halothane also had affects like nitrous oxide and caused degeneration in the dividing cells and maturation phase (4-8).

In our study membrana propria showing different degenerative changes in all experimental groups exhibited more degeneration in the second group because of the long exposure time and all of these changes caused an increase in the total thickness of the membrana propria. Basal lamina which has a physiologic importance in the blood-testes barrier appears in a homogeneous structure normally. It may show a few invaginations towards the tubular epithelium. But basal lamina change especially in the 2nd group may have occurred due to the dosage and the duration of the halothane nitrous oxide combination in our study. However the restoration of normal structure of membrana propria after stopping exposure may show the reversibility of the changes. But this can not be interpreted as an ultrastructural praperty in anesthetic exposure. As a matter of fact similar degenerative changes were reported in membrana propria in some testicular pathologies (11,14,16).

The first reaction in seminiferous tubules to anesthetic gases is seen in Sertoli cells. Electron density masking cytoplasmic organelles in the 1st group, dense phagocytic remnants occupying a wide area in the cytoplasm in the 2nd group and centrioles near the nucleus in the 3rd group were seen interestingly in the Sertoli cells. Sertoli cells supporting spermatogenic cells and having phagocytic characteristics are also responsible for the nutrition of the spermatogenic cells. Degeneration in the cytoplasmic organelles (SER vacuolization, degenerated mitochondria and large lipid droplets) are findings of the depraved cell metabolism. However in the 3rd group the appearence of centroiole in the cytoplasm shows that Sertoli cells can divide when needed. Thus it can be said that the Sertoli cells are affected as a result of chronic exposure to halothane-nitrous oxide combination having mutagenic effect on the dividing cells and in the maturation phase. Although it was recorded that Sertoli cells were resistant and spermatogenic cells were sensitive in the

studies done with inhalation anesthetics (2-6). Sertoli cells were the cells giving the first reaction. Thus spermatogenic affection occurred in our study. In fact Kaya (12), Kaya and Turkyilmaz (13), reported similar results and Kaya and Turkyilmaz (13) showed that Sertoli cells were affected firstly and might divide in reaction to pathologic changes affecting testicular structure when its is necessary.

it is interesting that spermatocytes and spermatide showed degenerative changes causing narrowing of tubular epithelium in the 2nd group. Pyknosis in the spermatocytes and spermatids may result in insufficient amount of spermatozoa in the testes. The developing spermatids with two nuclei showing acrosomal abnormalities may cause the occurrence of the spermatozoa having an abnormal head cap, two heads or two tails. Degenerative changes in the spermatids may be due to mutagenic effect of halothane-nitrous oxide combination. In fact it was reported that chronic exposure to inhalation anesthetics might inhibit spermatogenesis and thus cause a decrease in the number of spermatids and spermatozoa (2-7,9,13). Wharton et al. (9) reported that mutagenic effect occurred in male mouse offoprings exposed to subanesthetic and anesthetic concentrations of halothane. So the personnel working in the operating room were reported as risk group in respect to congenital anomalies and spontaneous abortus in USA and UK(6). In our study degenerative changes increased as the duration of exposure to inhalation anesthetics prolonged. It was reported that the toxic effect of halothane and nitrous oxide on male reproductive system was reversible (2). The presence of normalized tubules in the 4th group support this assumption. Spermatogenic cycle lasts for 35 to 36 days in mice (10). So the real reversible changes cair only be seen after 36 days. In our study because of high mortality rate in mice, we book samples in the 15th and 20th days to examine reversibility considering that diploid germ cell divide by mitosis every 9 days. Although degenerative changes occurred during exposure to anesthetic gases were normalized, heavy degeneration in some tubules suggests that spermatogenesis was inhibited for a long time. As a matter of the Kripke et al. (2) suggested that the decrease in the weight of the testes occurring during exposure to anesthetic gases was normalized 3 days after stopping exposure.

Leyding cells located in the interstitium are responsible for the synthesis and secretion of testosterone needed for spermatogenesis. Degeneration seen in the cyloplasmic organelles in the 1 and 2nd groups may be a result of exposure to halothane-nitrous oxide combination. On the other hand the presence of a few lipid droplets in Leyding cell cytoplasm, mature multiple Golgi complexes, normal SER and mitochondria in the 4 group suggest that the changes are reversible. Athough there are some reports that Leyding cells are also resistant to anesthetics like Sertoli cells (2,5), our findings show that Leyding cells had degenerative changes in response to anesthetic gases and were affected by anesthetics.

In conclusion halothane-nitrous oxide combination affectes Sertoli celis in testis firstly and thus spermatogenic cells cause the formation of abnormal spermatoza, but these changes are reversible.

Halotan - nitröz oksit kombinasyonunun fare testisleri ince yapısı üzerine etkisi

10-14 hafta arası yaşta, 40-50 gr. ağırlığında 30 adet erkek Albino fare rastgele seçildi. Birinci grup farelerin 5 gün, 2. grup farelerin 10 gün süreyle haiotan-nitröz oksit kombinasyonuna maruz bırakıldığ, 3. ve 4. grup farelerin 10 gün haiotan-nitröz oksit kombinasyonuna maruz bırakıldıktan sonra gazlar kesilerek aynı ortamda 3. grup farelerin 5 gün, 4. grup farelerin ise 10 gün daha yaşatıldığı bu çalışmada elde edilen testis biopsi materyalleri Zeiss E. M. 10 B elektron mikroskobuyla incelendi.

Birinci deney grubunda ansstezik gazlara ilk tepki farklı elektron denslikteki Sertoli hücrelerinde görüldü. Bazı Sertoli hücreleri organelleri maskeleyecek derecede elektron dens hale gelmişti. İkinci grupta bu hücrelerin sitoplazmalarında yaygın SER vakuolizasyonu ve iri, dens fagositik artıklar gözlendi. Üçüncü grupta ise Sertoli hücre sitoplazmasında sentriollerin varlığı dikkati çekti. İkinci deney grubuna ait mikrograflarda membrana propriada total kalınlık artışı, spermatositlerde piknoz, ortak ve ayrı akrozomlu çift çekirdekli spermatidler, lümende immatür ve piknotik spermatidler gözlendi. Leydig hücre sitoplazması da tamamen dejenere olmuş mitokondriyonlar, artmış lipid damlacıkları sergilemekteydi. Dördüncü grup mikrog raflarda membrana proprianın, seminiferöz tûbüllerin ve interstisyumun bazı tübüller dışında hemen hemen tamamen normal yapısını kazandığı görüldü.

Düşük doz haiotan-nitröz oksit kombinasyonunun testiste ilk önce Sertoli hücrelerini etkilediği, buna bağlı olarak da spermatogenik yıkımlanmanın oluştuğu, ancak bu dejeneratif değişikliklerin reversibl olduğu kanısına varıldı. [TurkJ Med Res 1993; 11 (6): 252-260]

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