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Research Article

Investigation of the Effects of Pre-Incubation Formaldehyde Fumigation on the Tracheal Epithelium of Chicken Embryos and Chicks

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Abstract: This study aimed to evaluate the effects of pre-incubation formaldehyde fumigation on the tracheal epithelium of chicken embryos and chicks. Throughout the study pre-incubation formaldehyde fumigation was applied to 18-day-old embryos and 1-day-old chicks only once, at 1 of 2 different concentrations (3×, 42 ml of formalin and 21 g of potassium permanganate per m^3 and 4×, 56 ml of formalin and 28 g of potassium permanganate per m^3) for 1 of 2 different durations (20 min and 40 min). Tracheal samples were taken from 18-day-old embryos and 1-day-old chicks, and the tracheal epithelial cells were examined with transmission electron microscopy (TEM). According to TEM, after fumigation cilia in the epithelial cells were shorter and fewer in number, and vacuolisation, swelling of the mitochondria, and spoiling of cristae were observed in the subjects, which varied according to fumigation concentration and duration.

Key Words: Chick, chicken embryo, disinfection, formaldehyde, tracheal epithelium, transmission electron microscopy

İnkübasyon Öncesi Formaldehit Fumigasyonunun Tavuk Embriyoları ve Civcivlerin Trake Epiteli Üzerine Etkilerinin Araştırılması

Özet: Bu çalışmada, inkübasyon öncesi formaldehit fumigasyonunun tavuk embriyosu ve civcivlerin trake epiteli üzerine olası etkileri incelenmiştir. Formaldehit fumigasyonu inkübasyon öncesi iki farklı doz (m^3 için 42 ml formalin ve 21 g potasyum permanganat = 3× ve 56 ml formalin ve 28 g potasyum permanganat = 4×) ve sürede (20 dak ve 40 dak) bir kez uygulanmıştır. Trake örnekleri 18 günlük embriyo ve 1 günlük civcivlerden alınmıştır. Trake epitel hücreleri transmisyon elektron mikroskobu ile incelenmiştir. Bu incelemelere göre 18 günlük embriyo ve 1 günlük civciv trake epitel hücrelerinde konsantrasyon ve fumigasyonun uygulama süresine bağlı olarak, epitel hücrelerinin sillerinde kısalma ve azalma, vakuol oluşumu, mitokondrilerde şişme ve kristalarında bozulma tespit edilmiştir.

Anahtar Sözcükler: Civciv, tavuk embriyosu, dezenfeksiyon, formaldehit, trake epiteli, transmisyon elektron mikroskopisi

Introduction

Disinfection is vital in commercial poultry hatcheries in order to protect against disease and reduce the number of pathogens in the hatchery environment. An egg is sterile during its formation, but microbial contamination of the eggshell occurs during the passage through the cloaca and following oviposition (1). Eventually, bacteria penetrate the shell and infect the embryo, resulting in the inability to hatch, poor quality chicks or poults, and infection in the growing birds (2,3).

Formaldehyde is used as a disinfectant in commercial poultry hatcheries to reduce the number of pathogens (such as *Salmonella, Escherichia coli*, and *Pseudomonas*) in the hatchery environment (4). Formalin (40% formaldehyde) is mixed with the oxidising agent, potassium permanganate, to generate a gas. Eggs are then exposed to this gas in a tightly closed cabinet or room (5). Although formaldehyde is a very toxic gas, it is commonly used because it is easy to apply and is an effective disinfectant; however, there may be undesired

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consequences when working with this toxic gas (6). Sander et al. (4), Fauziah et al. (7) and Zulkifli et al. (8) reported that exposing hatching chicks to 130 ppm of formaldehyde vapour during the last 3 days of incubation damages the chicks' tracheal epithelium.

Formaldehyde, used in hatcheries as a means of sanitation, produces ciliostasis and causes blunting and surface blebbing in the tracheal cilia of exposed chicks (9). Excessive mucus production, matted cilia, and areas of deciliation may result in inadequate mucociliary action (10).

Noxious gases act as irritants to the delicate tissues of the upper respiratory system (10,11). Formaldehyde impairs mucociliary mechanisms and affects the flow of mucus (10). Exposing chicks to formaldehyde gas during pipping was shown to cause ciliostasis and abnormal morphology, which appear as blunted cilia with apparent blebs in the ciliary wall with scanning electron microscopy (9).

The purpose of the present study was to examine the tracheal tissues of chicken embryos (18 days old) and chicks (1 day old) exposed to pre-incubation fumigation with formaldehyde at 2 different concentrations and 2 different durations. Transmission electron microscopy (TEM) was used to examine the ultra-structure of the tracheal epithelial cells.

Materials and Methods

Chickens

The study included 1464 broiler hatching eggs. All eggs were taken from the Poultry Research Institute, Ankara, Turkey, where the formaldehyde fumigation and incubation were conducted.

Formaldehyde fumigation

Pre-incubation fumigation was applied only once, at 1 of 2 different concentrations, (3×, 42 ml of formalin + 21 g of potassium permanganate per m^3 and 4×, 56 ml of formalin + 28 g of potassium permanganate per m^3) for 1 of 2 different durations (20 min and 40 min). One control group and 2 experimental groups were used in this study. The control group and each experimental group contained 366 fertilised eggs each. The first experimental group contained fertilised eggs exposed to formaldehyde fumigation with 42 ml of formalin (40% HCHO) and 21 g of potassium permanganate per m^3 (3×). Fumigated fertilised chicken eggs in the second experimental group were exposed to 56 ml of formalin (40% HCHO) and 28 g of potassium permanganate per m^3 (4×). Each experimental group was divided into subgroups denoting 2 different fumigation periods (20 min and 40 min). The fumigated fertilised chicken eggs were then incubated at 38-40 °C and 70% relative humidity.

Tracheal Collection and Processing

Tracheal samples were taken from 18-day-old embryos and 1-day-old chicks. In all, 10 tracheal tissue samples from each group were randomly collected for light microscopy and TEM. For light microscopy the samples were fixed in Bouin's solution, dehydrated, and embedded in paraffin. Then, 4-µm sections were stained with haematoxylin and eosin (H&E), and observed (12). For TEM, tissue samples were fixed in 3% glutaraldehyde (pH 7.2). Following the fixation, the tissues were rinsed 3 times for 30 min (3 × 30 min) in sodium phosphate buffer (pH 7.2), and then post-fixed for 90 min in 1% osmium tetroxide, again in the sodium phosphate buffer (pH 7.2). After fixing, the tissue samples were dehydrated in an ethanol series. After dehydration in 100% ethanol, the tissue samples were embedded in araldite. Ultra-thin sections were cut with a glass knife and stained with 2% uranyl acetate and lead citrate (13). The grids were viewed and photographed with a Jeol 100 CX II transmission electron microscope operating at 80 keV.

Results

There were no pathological changes observed in the tracheal epithelium of the 18-day-old embryos (Figures 1 and 2) or 1-day-old chicks in the control group (Figures 3 and 4).

With TEM, pathological changes were observed in the tracheal epithelium of 18-day-old embryos and 1-day-old chicks exposed to fumigation. In the 18-day-old embryos exposed to formaldehyde fumigation at the 3× concentration for 20 min, decreases in the number of cilia and swelling of the mitochondria were observed in the tracheal epithelium (Figures 5 and 6). When the group was fumigated for 40 min, swelling of the mitochondria and spoiling of the cristae increased (Figure 7). Similar findings were observed in 18-day-old embryos fumigated with the 4× concentration for 20 min. Vacuolisation in

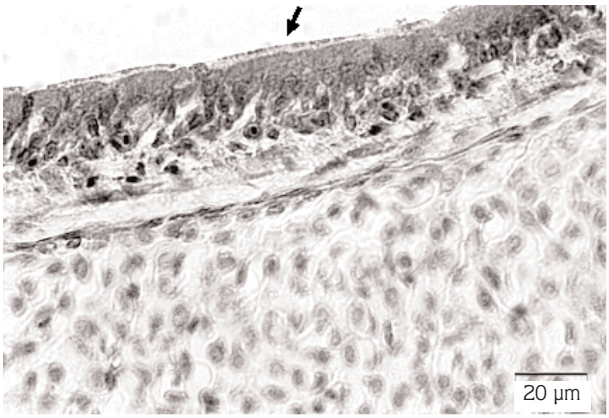

Figure 1. Paraffin tracheal section of an 18-day-old embryo in the control group (arrow points to cilia) (H&E).

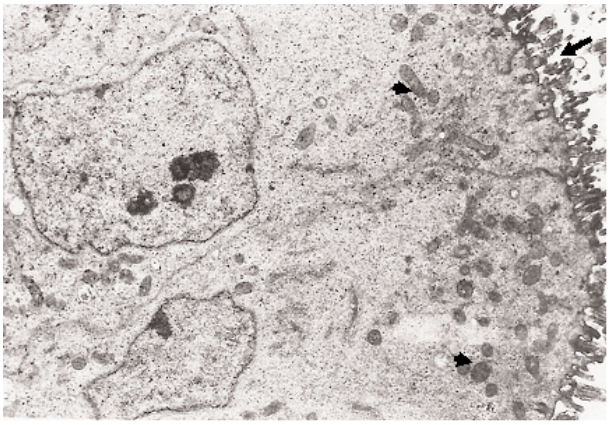

Figure 2. TEM of tracheal epithelium cells of an 18-day-old embryo in the control group shows mitochondria (arrow head) and cilia (thick arrow) (4800×).

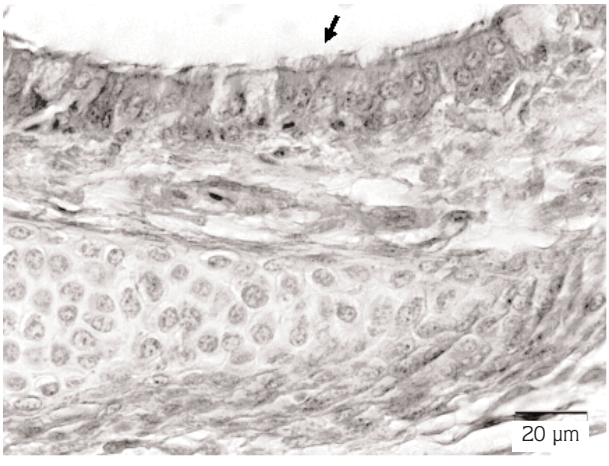

Figure 3. Paraffin tracheal section of a 1-day-old chick in the control group (arrow points to cilia) (H&E).

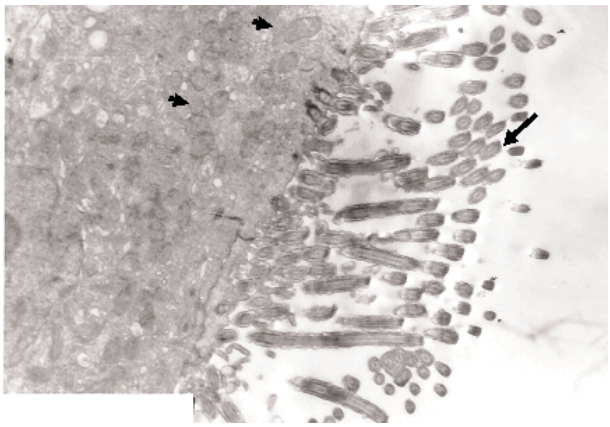

Figure 4. TEM of tracheal epithelium cells of a 1-day-old chick in the control group shows mitochondria (arrow head) and cilia (thick arrow) (7200×).

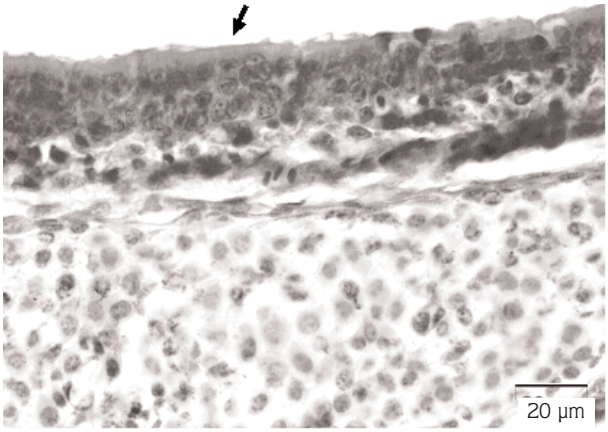

Figure 5. Paraffin tracheal section of an 18-day-old embryo exposed to 3× fumigation and for 20 min shows epithelial layer with deciliated cells (arrow) (H&E).

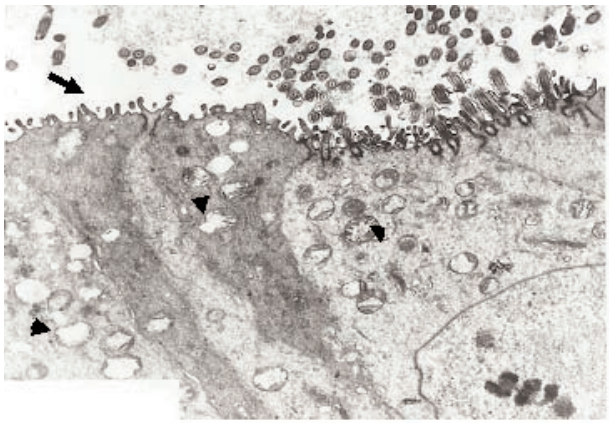

Figure 6. Swelling of mitochondria (arrow head) and decreased number of cilia (thick arrow) in the tracheal epithelium of an 18-day-old embryo exposed to the 3× fumigation for 20 min (TEM, 5800×).

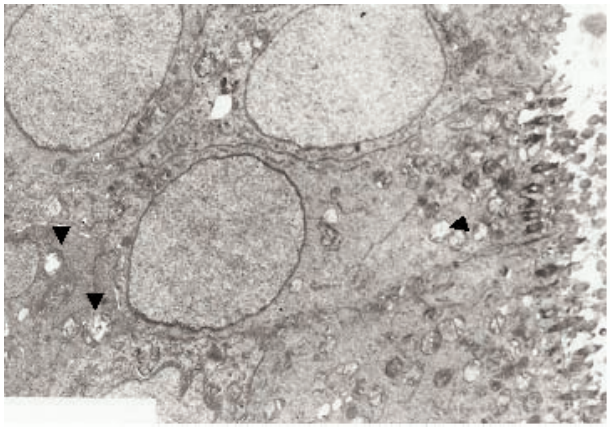

Figure 7. Swelling of mitochondria and spoiling of cristae (arrow head) in the tracheal epithelium of an 18-day-old embryo exposed to the 3× fumigation 40 min (TEM, 4800×).

the epithelia cells was also observed in the 18-day-old embryos exposed to the same concentration for 40 min (Figure 8).

Similar findings were noted in 1-day-old chicks exposed to formaldehyde fumigation at the 3× concentration for 20 min; that is, decreases in the number of cilia, swelling of the mitochondria, and spoiling of the cristae were observed. In addition to the swelling of the mitochondria and spoiling of the cristae, exposure to 3× fumigation for 40 min caused vacuolisation and loss of cilia (Figures 9 and 10). As the fumigation period increased, vacuolisation, mitochondrial defects, and lysosomal vacuolisation also increased, with a shortening of cilia and regional cilia losses (Figure 11).

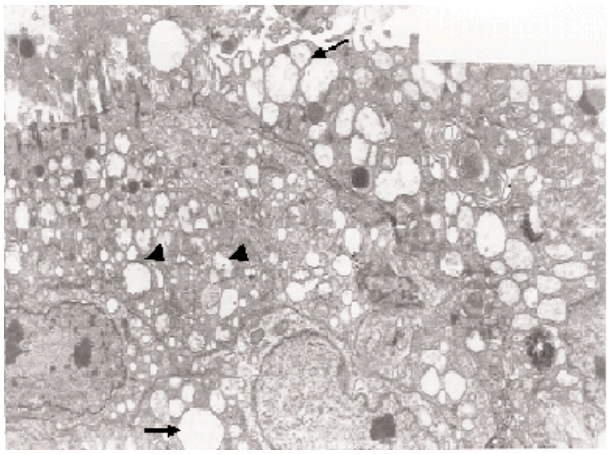

Figure 8. Swelling of mitochondria, spoiling of cristae (arrow head), and vacuolisation (arrow) in the tracheal epithelial cells of an 18-day-old embryo exposed to the 4× fumigation for 40 min (TEM, 4800×).

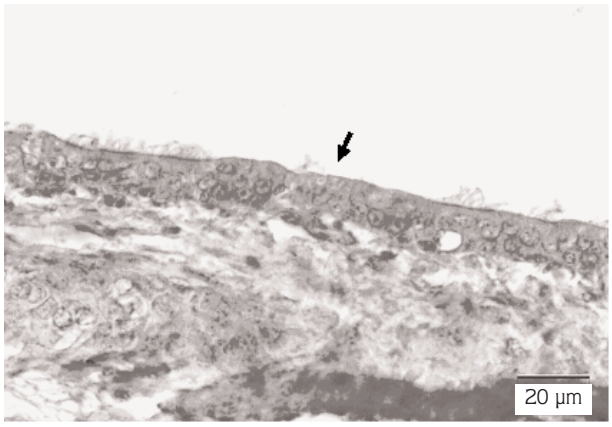

Figure 9. Paraffin tracheal section of a 1-day-old chick exposed to the 3× fumigation for 40 min shows epithelial layer with deciliated cells (arrow) (H&E).

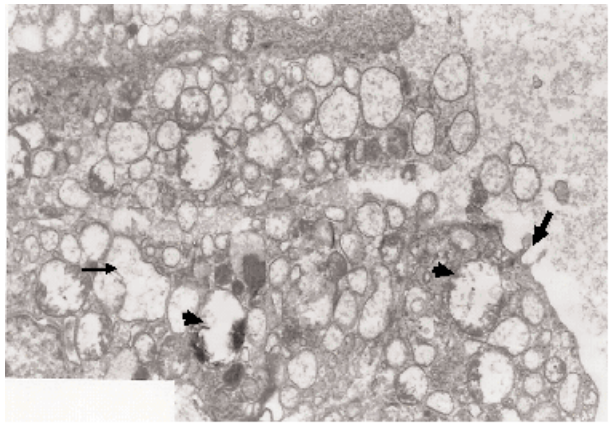

Figure 10. Loss of cilia (thick arrow), vacuolisation (arrow), swelling of mitochondria, and spoiling of cristae (arrowhead) in the tracheal epithelial cells of a 1-day-old embryo exposed to 3× fumigation for 40 min (TEM, 7200×).

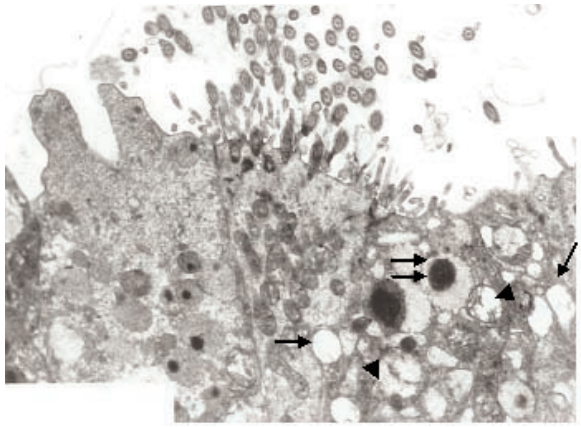

Figure 11. Vacuolisation (arrow), mitochondrial defects (arrowhead), and lysosomes (double arrow) in the tracheal epithelial cells of a 1-day-old embryo exposed to 4× fumigation for 40 min (TEM, 7200×).

Discussion

This study was designed to evaluate the effects of pre-incubation formaldehyde fumigation on the tracheal epithelium of chicken embryos and chicks. Many studies have been conducted to determine the effects of formaldehyde fumigation on chicken embryos during the final 3 days of incubation (4,8,14). Zulkifli et al. (8) reported that excessive accumulation of mucus, matted cilia, loss of cilia, and sloughing of the epithelium was observed in chicks administrated formaldehyde fumigation during the last 3 days of incubation at 23.5 ppm. Sander et al. (9) reported that exposing chicks to formaldehyde gas during pipping caused ciliostasis and abnormal morphology, which is seen with scanning electron microscopy as blunted cilia with apparent blebs in the ciliary wall.

In the present study, TEM revealed shortening and loss of cilia in the tracheal epithelium of 18-day-old embryos and 1-day-old chicks. Eggs were exposed to pre-incubation formaldehyde fumigation, unlike in previous studies. Tracheal samples taken from 18-day-old embryos and 1-day-old chicks were investigated using TEM, and damage to the tracheal cells was observed. According to TEM investigation, shortening and loss of cilia in the epithelial cells, vacuolisation, swelling of the mitochondria, and spoiling of their cristae were present in both 18-day-old embryos and 1-day-old chicks. Extending the fumigation period caused increases in these effects.

An important factor in the effect of formaldehyde on the tracheal mucosa is the dissolution of the gas in secretions. Formaldehyde dissolved in mucous secretions causes a pH shift toward acidity (10) and these changes in pH cause damage to the membrane structure and ciliary activity. This study also shows that liquid and ion alteration may cause mitochondrial damage and vacuolisation in epithelial cells.

Consequently, we conclude that pre-incubation formaldehyde fumigation negatively affects tracheal epithelium cells in chicken embryos and chicks.

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