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THE HAEMATOLOGICAL CHANGES AND HEARTBEAT VARIATION IN WHITE LEGHORN EMBRYOGALLUS GALLUSDOMESTICUSEXPOSED TO HIGH DECIBEL SOUND

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Keywords: *Noise pollution, chick embryo, apoptosis.*

Abstract

Stress during theembryonic phase may cause many changes in their physiology and morphology. Exposure to noise can influence the developmental stage and also the cognitive ability of the new-born baby. White Leghorn (*Gallus gallusdomesticus*) chick embryo was taken as test model and was exposed a high level of noise. In the present study results showed growth retardation, high heart rate and apoptosis in white leghorn embryoca used due to 96 -decibel noise exposure.

Introduction

Noise can be considered as an environmental stress and unwanted sound or sound above 55 db is considered as a noise pollution. Exposure of high decibel of sound has an adverse effect on human health such as sleep-apnoea, stress, and cardiovascular diseases are related to the high level of sound (Muralidharan*et. al.*, 2018).It has been observed that noise is a major cause to neurodegenerative changes in brain (Kesar, 2014). Sound between the ranges of 160 to 180 dB has a hazardous impact to the inner ear (Häusler, 2004). Kim et al., (2013) Suggests that during the developmental period noise work as an important factor and it influences the brain development and urogenital disorder. Chicken embryos are very susceptible to stress (Epple*et al.*, 1997). High level of noise can increase increased brain acetyl cholinesterase activity and plasma corticosterone and adrenocorticotropic hormone levels (Kesar, 2014). According to Weinstock (1997), the effect of prenatal stress are as follow: "elevation of gluco-corticoids, increased emotionality, increased hypothalamic-hypophyseal-adrenal responsiveness, increased number of glucocorticoid receptors in the hypothalamus and hippocampus, delays in early motor development, greater hypothalamic β-endorphin, feminization of males, and masculinization of females". Embryonic development plays an important role in the performance and biological values of adult birds(pawalak andNiedziólka, 2004). According to Sanyal*et. al.*, (2013) high level of noise can increases noradrenaline level in plasma and positively modulates spatial orientation, learning and memory of chick. The present study aimed to investigate the effect of noise pollution on chick embryo in its developmental stage.

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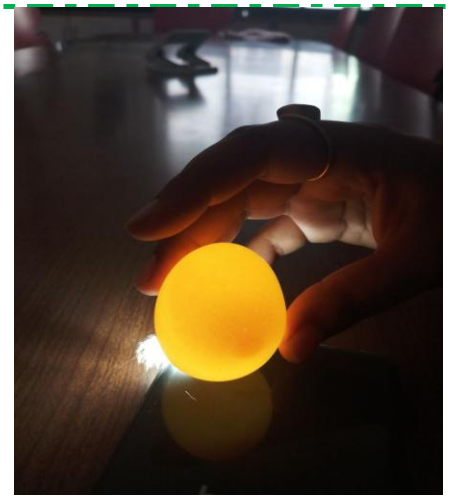

Figure 1: Candling method to observe egg

Material and Methods

Material: Egg of White leghorn (*Gallus gallusdomesticus*), a common hen, were collected from Aarey colony, Goregaon, Mumbai. 50 eggs were selected for the experiment and they were equally divided into two groups i.e., control and experimental.

Incubation: Eggs from both the group were incubated in two separate incubators at the temperature 37.5± 1 ^{o}C and humidity was maintained between 55% - 60%.Eggs were rotated/ tilted two times daily at regular interval to mimic the natural condition throughout the incubation period of experiment to provide proper growth of embryo. Photoperiod of 12:12 hour day: night cycle were maintained manually.

Experiment: Experiment was carried out for 12 days and observations were done on day 4, 9, and 12. Initial weight of egg was determined to be 33g to 36g.The parameters for the experiment were chosen are as follows: weight of eggs, weight of embryo, heart rate and blood cell profile. Heart beat was calculated manually. Recorded train sound of 96dB was givenfor four hours every day only for experimental group throughout the study period..Candling method was used for the observation of embryo (Figure 1).

Results and discussion

During experiment out of 50, ten eggs were found unfertilized so 20 eggs (10 for control and 10 for experimental group) were removed to maintain the consistency of the experiment.The results obtained are presented in Table 1 to 3.

A significant increase was observed in weight of eggs from control group (Table 1; Figure 7), as compared to exposed group (Table 1; Figure 7).

Embryonic weight increased significantly in control group on day 4, 9, (Figure 2) and day 12. Weight of embryo increased in experimental group (Table 2; Figure 3 and 8). The increase in weight in experimental group was low as compared to control group (Table 2; Figure 8).

Heart rate was observed increased in both the control and experimental group (Table 3; Figure 9). The increase in heart rate was more significant in experimental group (Table3; Figure 9).

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For the blood cell morphology blood was taken on day 12 from both the groups. Staining was done with the leishmn's stain. Pyknosis, karyorhhexis and karyolysis was observed in experimental group (Figure 5). High number of neutrophils and macrophages were observed in experiment group (Figure 5) as compared to control group (Figure 4 and 5).

Table 1: Weight of eggs of white leghorn control and experimental group measured on 4,9,12th day

	Control (mean ± SE)	**Experiment (mean ± SE)**
Day 4	35 ± 0.365	34 ± 0.516
Day 9	36.8 ± 0.476	34 ± 0.365
Day 12	45 ± 0.365	39.6 ± 0.611

Table 2: Weight of embryo of white leghorn control and experimental group measured on 4,9,12th day

	Control (mean ± SE)	**Experiment (mean ± SE)**
Day 4	0.05 ± 0.002	0.048 ± 0.003
Day 9	1.55 ± 0.003	1.24 ± 0.015
Day 12	5.022 ± 0.007	1.522 ± 0.003

Table 3: Heart rate per minute of white leghorn control and experimental group measured on 4,9,12th day

	Control (mean ± SE)	**Experiment (mean ± SE)**
Day 4	113.2 ± 0.305	95± 0.258
Day 9	114.2 ± 0.476	144.4 ± 0.553
Day 12	111.6 ± 0.20	147.4 ± 0.711

Figure 2: 9 days embryo from control group

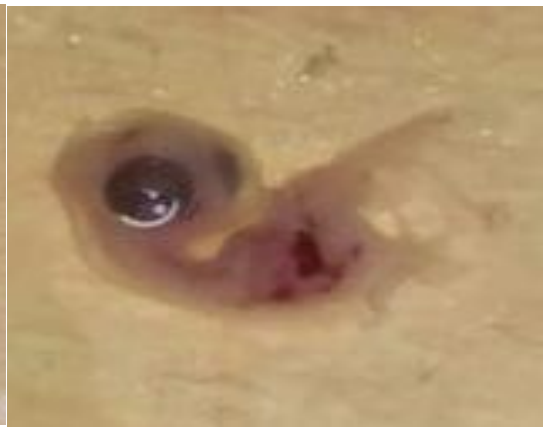

Figure3: 9 days embryo from experimental group

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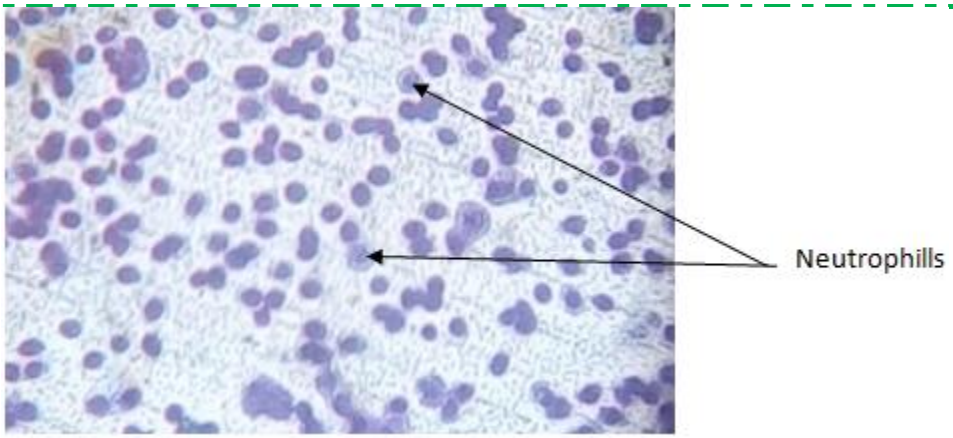

Figure 4: Blood smear from control group

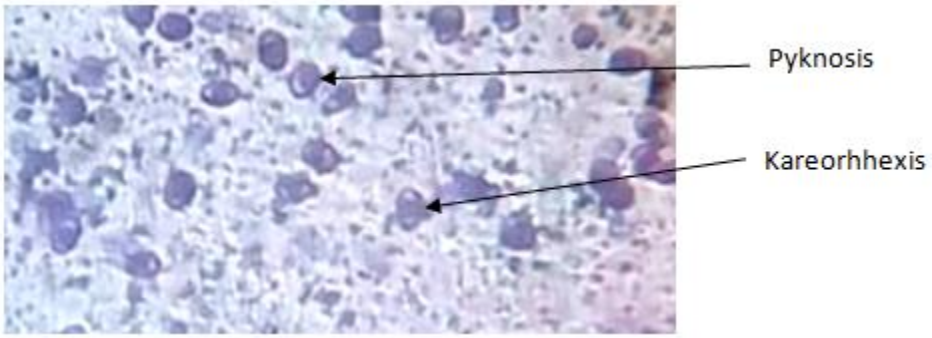

Figure 5: Presence of necrotic cells in experimental group

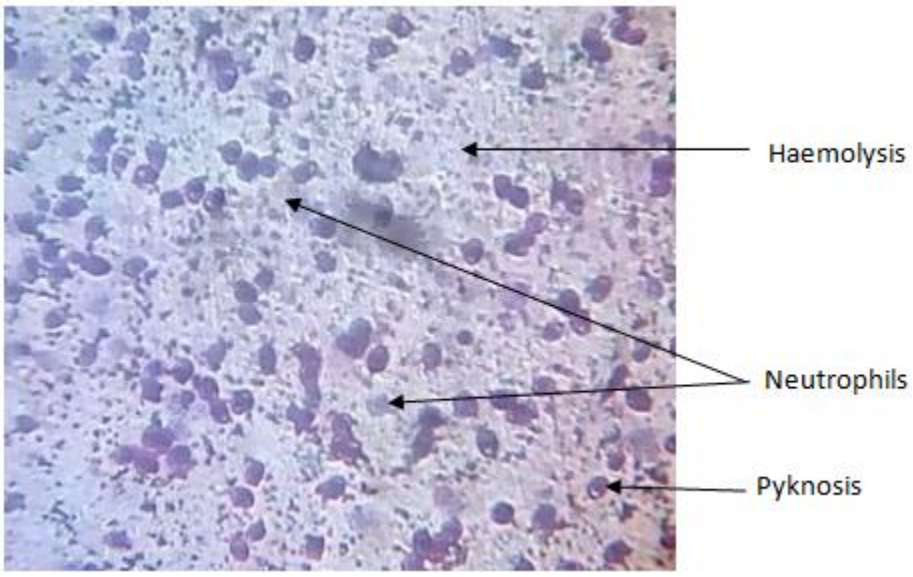

Figure 6: presence of haemolysis, necrosis and large no. of neutrophills

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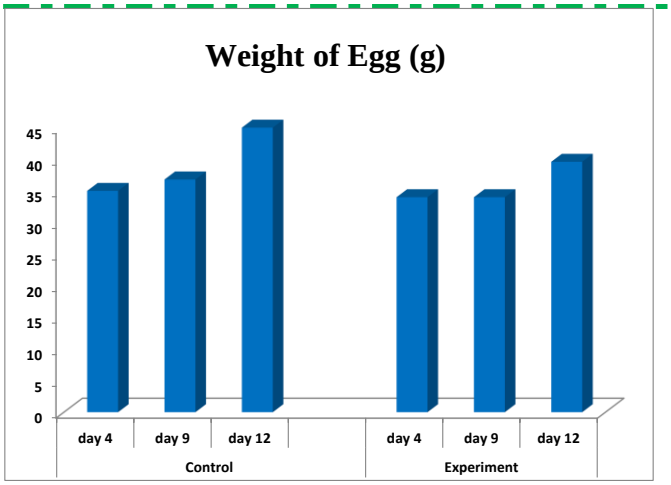

Figure 7: Weight of egg (g)

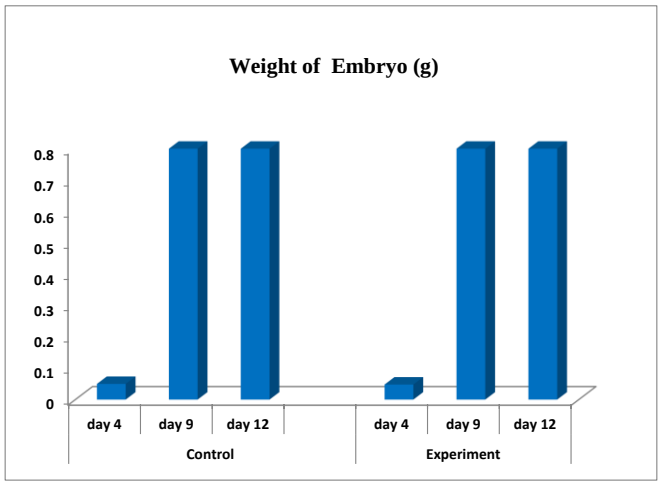

Figure 8: Weight of embryo (g)

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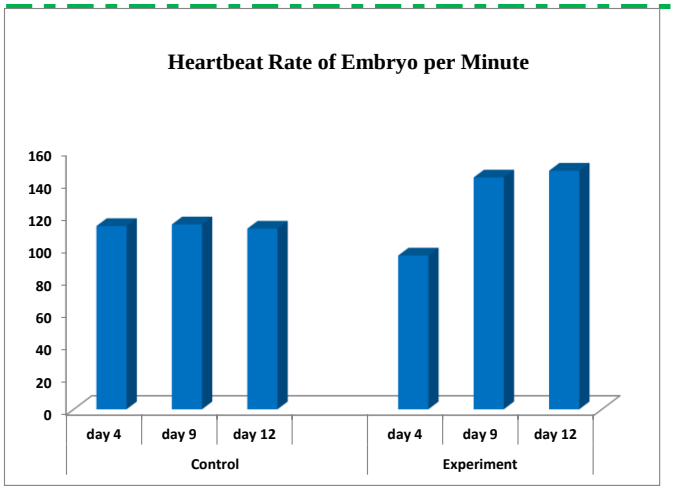

Figure 9: Heart rate of embryo per minute

Development of foetus is affected by its prenatal environmental condition (Kim *et. al.*, 2013; Tate *et. al.*, 2016).The present study investigates the effect of noise exposure on growth of chick embryo. Auditory stimuli to chick were given 96 db at the fix time interval. This level of sound is considered as noise, therefore, in present research work the sound given to the chick embryo is referring as noise.

In present study the initial weight of egg and embryo was almost same for control and experimental group ranging between 33 to 36 g. The weight of egg and embryonic weight were observed significantly less in experimental group after post hatching day 4, 9, and 12. It may be due to stress. The present results are supported by the findings of Kesar (2014). According to Kesar (2014) growth retardation is linked to the excessive sound exposure during the sensitive time period of embryonic development. Similar observation was evident by Kim *et. al.*, (2006) in rats, they suggested that decrease in body weight may be due to decreased neurogenesis in hippocampus.

The development of chick is controlled by endocrine mechanism (Crossley*et. al.*, 2003). In chick embryo cardiovascular function is maintained by adrenergic receptor stimulation (Crossley and Altimiras, 2000). According to Ruijtenbeek (2002) causes of embryonic stress are malnutrition and chronic hypoxia.Crossley II *et. al.*, (2003) suggested that heart rate of chick embryo is influenced by muscarinic actions on the heart and the adrenergic effects on the vasculature.Sanyal*et. al.*, (2013) reported that increase in plasma ,adrenaline level and changes in expression of synaptic protein in hippocampus are related to noise stimulation.Epple (1997) suggested that the chicks in embryonic period are very susceptible to stress. In present study heart rate of chick embryo was found to be increased in experimental group. It may be due to stress bynoise pollution.

Noise pollution has a significant impact on blood and it is responsible for increased stress, tissue dysfunction and hormonal changes (Mohammadi*et. al.*, 2016, Münzel et al., 2017). Earlier studies show that noise pollution has an impact on RBC, WBC, haemoglobin and haematocrit of blood cells of mice (Sabahi and Moradi, 2002). Other studies show that due to noise exposure metabolic activity increases and blood flow decreases and results in formation of free radical by the cellular redox state (Evans and Halliwell, 1999). This free radical can generate by different mechanism such as a by-product of mitochondrial respiration (ROS or Reactive oxygen species), environmental contamination or UV radiation (Darrat*et. al.*, 2007). These reactive oxygen species can damage cellular pathway and lead to apoptotic pathway, causing cell death and permanent cell damage (Campbell *et. al.*, 2003).Mitochondria play a important role in apoptosis, or programmed cell death(PCD). During mitochondrial dysfunction pro-caspases, cytochrome C, apoptosis-inducing factor (AIF), and apoptotic protease-activating factor-1 (APAF-1) are released into the cytosol where they form a complex of cytochrome C, APAF-1 and caspase 9. This

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complex activates the caspases and resulting into the apoptotic cell death (Kannan and Jain, 2000). Föller*et. al.*, (2008) reported eryptosis (death of erythrocytes), by cell shrinkage and membraneblabbing.In present study, blood cells from experimental group show the apoptic cells, haemolysis, cell blabbing, cell shrinkage and large number of neutrophils and macrophages. These results are supported by the observation of Föller and colleagues (2008).

In present paper, body weight of chick embryo and egg weight were found decreased whereas, heart rate was found to be significantly increased in noise exposure group i.e., experimental group. The decrease in weight may be associated with impairment of hippocampus activity. Apoptotic cell death was recorded in experimental group which can be attributed to stress generated by exposure to noise.

Thus, the noise related stress can causes the growth retardation, impair development of body, and apoptotic cell death in blood cell of chick embryo during prenatal stage of development.

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