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Effect of Exposure to Extremely Low Frequency Electromagnetic Fields (50 Hz, 0.5 mT) During Incubation on Hatchability, T_3 and T_4 Levels in Newly-hatched Chicks

Alireza Lotfi and Mohammad Narimani-Rad
Ilkhchi Branch, Islamic Azad University, Ilkhchi, Iran

Corresponding Author: Alireza Lotfi, Ilkhchi Branch, Islamic Azad University, Ilkhchi, Iran

ABSTRACT

The aim of present study was to determine the effect of intermitted exposure to Extremely Low Frequency (ELF) and Electromagnetic Fields (EMF) during different periods of incubation on embryonic mortality, total hatchability and thyroid hormones level in hatched chicks. Experimental groups were classified as group 1 (control), with normal incubation process and without any exposing to EMF; group 2 included eggs exposed to ELF-EMF, 2 h daily for 0-7 day of incubation; group 3 included eggs exposed for 2 h daily from day-8 to -14 of incubation; group 4; included eggs exposed from day-15 to -21 of incubation and group 5 included eggs exposed from day-1 to -21 of incubation (whole incubation period). Incubation condition with exception to EMF exposing was similar for all groups. After observations for hatching characterizes, the blood samples were collected. The samples were centrifuged and serum was obtained for determination of the T_3 and T_4 level. Exposure to EMFs didn't have similar effect on hatchability; the groups 2 and 3 that exposed for a stage had significant lower hatchability in comparison with group 1 (control) and group 5. Group 3 had highest mid-embryonic mortality rate. The late-embryonic mortality was high in control group. The group 5 had highest early-embryonic mortality rate. There is not any significant difference for T_3 concentrations between groups. Level of T_4 is significantly increased for groups 2 and 3, when it compared with control groups ($p<0.05$). It was concluded that intermitted exposure to ELF- EMF during 2nd or 3rd week of embryonic life can cause considerable mortality rate and hatchability decreases. Although exposure to EMF entire incubation period or at first week couldn't has significant effect on hatchability. Exposing to ELF-EMF during 1-14 d of incubation caused T_4 level increases, whereas T_3 level was not affected by EMF.

Key words: Electromagnetic fields, exposure, incubation, chicken embryo, thyroid hormones

INTRODUCTION

Nowadays, Electromagnetic Field (EMF) and its hazardous or beneficial biological effects are subject of so many studies on human and animals. Exposure to EMF was studied in poultry at pre-incubation (Lahijani and Sajadi, 2004), during-incubation (Ingole and Ghosh, 2006; Batellier *et al*., 2008) or post-incubation (Cuppen *et al*., 2007).

The environmental factors that are most critical to the optimal development of the embryo are those that occur during the incubation and hatching processes. Any alterations in incubation environment influences the metabolism and growth of embryos with possible consequent at post-hatch life and affect finishing outcome especially in broiler type chicken via changes in the

efficiency of nutrient metabolism and utilization (Shafey, 2004; Shafey *et al*., 2007). At current decade, researchers have done focused on other environmental factors in hatching process such as light color (Shafey, 2004), electric fields (Shafey *et al*., 2007) and Electromagnetic Fields (EMF) (Ingole and Ghosh, 2006; Batellier *et al*., 2008).

Embryonic exposure to EMFs during incubation had detrimental effects on embryo development and hatchability (Pisiriciler *et al*., 2001; Batellier *et al*., 2008). Especially higher frequencies of EMF such as 900 MHz (emitted by cellular phones) can affect hatching results with increasing embryonic mortality and decreasing hatchability, also has post-hatch negative effect on chicken viability (Zareen and Yunuskhan, 2008). In this regard, Batellier *et al*. (2008) had stated that group of eggs with exposure to 900 MHz EMFs had significant high embryonic mortality in comparison with control group incubated in condition without any EMF.

The growth of the embryo, as measured by body weight, skeletal size, muscle growth and growth of cartilages and bone, is greatly influenced by thyroid hormones in late embryonic development (King and May, 1984). Thyroid activity of chicken is critical regulator for incidence of hatching. Drastic changes in peripheral thyroxin level are occurred before hatch (Decuypere *et al*., 1990). Studies on mammalian models show sensitivity of thyroid function and changes in T3- T4 hormones level in exposure to high and low frequency EMF exposure (Koyu *et al*., 2005; Shahryar *et al*., 2009; Anselmo *et al*., 2009; Hosseini *et al*., 2011).

Sechman *et al*. (2006) had EMF experiment entire incubation period, stated that ELF-EMF stimulates the activity of the thyroid gland and influences the hatching parameters.

Regardless to their study on bio-electromagnetic effect on chick embryonic activity for first time but in their study (Sechman *et al*., 2006) the sensitivity of chicken embryo to hazardous effect of EMF was not investigated at different stages of embryonic life. In other word, when the embryonic thyroid gland is vulnerable and affected by EMF? That is a question to makes background for present experiment with different experimental design and EMF exposure at different stages of embryonic life.

MATERIALS AND METHODS

Design and description of EMF emitter set: The EMF emitter designed for produce ELF-EMF with 50 Hz frequency and 0.5 mT intensity with using urban electric power. An adaptor 220 v to 110 v (10 A) was applied for minimizing thermal effect of EMF emitter coin (Fig. 1). The EMF emitter set including bobbin (80×10 cm), wires and metal nucleuses was put in the bottom of hatchery machine in a lacuna (Fig. 1).

Experimental groups, incubation and EMF exposing: Four hundred fifty fertilized eggs with similar weight were collected from commercial broiler breeder (Ross 308) farm. Experimental design was based on Completely Randomized Design (CRD) with five treatment, three replicate for each one and 50 eggs in each replicate. The experimental groups were included; group 1: control; had normal incubation process and without any exposing to EMF, group 2 includes eggs exposed to 50 Hz, 0.5 mT, 2 h daily for 0-7 day of incubation, group 3 includes eggs exposed to 50 Hz, 0.5 mT, 2 h daily from day-8 to day-14 of incubation, group 4 includes eggs exposed to 50 Hz, 0.5 mT, 2 h daily from day-15 to day-21 of incubation and group 5 includes eggs exposed to 50 Hz, 0.5 mT, 2 h daily from day-1 to day-21 of incubation (whole incubation period).

Hatchery temperature and humidity were regulated (37.8°C, 55% RH from day-1 to day-18 and 37.2°C, 70% RH from day-18 to day-21 or hatching time). At EMF exposing time, EMF set were

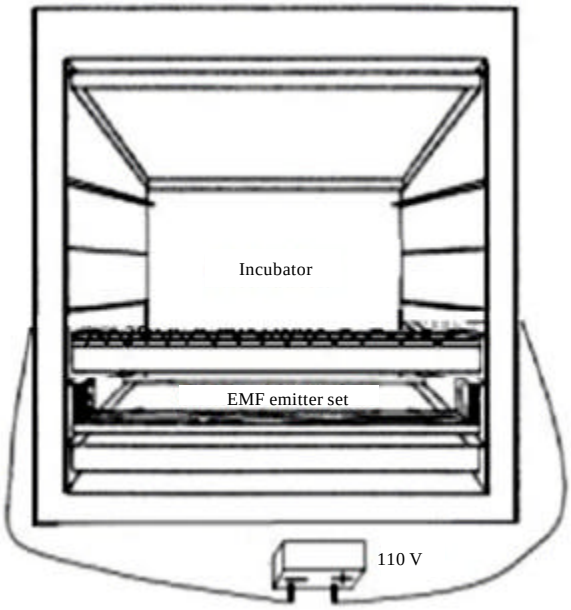

Fig. 1: Schema of incubator and EMF exposing condition

separated from setters (eggs in upper rows) via aluminum sheet coverage for avoiding any unfavorable exposure of other experimental groups. Also bottom of unexposed groups were covered with another aluminum sheet. At EMF exposing time (2 h daily) eggs were transferred to EMF emitter set (lacuna) and after exposing period, eggs were transferred to their rows (setter). Transfer of groups was done in 15 min for avoiding possible temperature change of incubation process (Fig. 1).

Observations and assay: At end of day-21, total hatchability was estimated as present of hatched eggs from total fertile eggs. The un-hatched eggs were broken and embryonic mortality was recorded as three different stages (early: 0-7 day, mid: 8-15 day and late: 16-21 day of embryonic life) via macroscopic observations and according to standard figures of chick embryo morphology.

The blood samples from each group were collected following chick decapitation. Blood samples were centrifuged and serum was obtained for determination of the T_3 and T_4 level by an electrochemiluminescence immunoassay (ECLIA) method.

Collected data were analyzed by SAS software Ver. 9.1 and Duncan multiple Tests were applied to find significant differences among means of groups.

RESULTS

According to Table 1, exposure to EMF in different stages of chick embryonic life didn't have similar effect on hatchability; the groups 2 and 3 that exposed for a stage (intermitted), had significant lower hatchability in comparison with group 1 (control) and group 5 (whole period). Group 3 (exposure at second week) had highest mid-embryonic mortality rate, in other words the embryo mortality was occurred at time of exposure (8-15 day). The late-embryonic mortality was high in control group. The group 5 (exposure for whole period) had highest early-embryonic mortality rate (Table 1).

Table 1: The embryonic mortality rate and total hatchability of eggs submitted to intermitted ELF-EMF during incubation

Groups	EMF duration	Early- embryonic mortality 0-7 day	Mid-embryonic mortality 8-15 day	Late-embryonic mortality 16-21 day	Total hatchability
1	Unexposed	9.03[a]	9.09[e]	81.87[d]	70.33[b]
2	50 Hz, 0.5 mT 0-7 day, 2 h daily	18.06[c]	18.06[d]	63.87[b]	70.97[b]
3	50 Hz, 0.5 mT 8-14 day , 2 h daily	12.33[d]	37.33[a]	50.33[c]	56.58[c]
4	50 Hz, 0.5 mT 15-21 day , 2 h daily	7.18[b]	21.71[c]	71.11[e]	56.10[c]
5	50 Hz, 0.5 mT 0-21 day , 2 h daily	28.34[e]	28.34[b]	42.23[a]	83.03[a]
p-value	-	0.0001	0.0001	0.0001	0.0001
SEM	-	0.196	0.182	0.233	0.578

Different letters show significant difference between experimental groups

Table 2: The effect of exposure to ELF-EMF during incubation on T_3 and T_4 hormones level in newly-hatched chickens

Groups	ELF-EMF duration	T_3 (ng mL^{-1})	T_4 (ng mL^{-1})
1	Unexposed	10.0	3.6[b]
2	50 Hz, 0.5 mT 0-7 day, 2 h daily	10.1	5.4[a]
3	50 Hz, 0.5 mT 8-14 day, 2 h daily	10.1	5.1[a]
4	50 Hz, 0.5 mT 15-21 day, 2 h daily	10.6	3.7[b]
5	50 Hz, 0.5 mT 0-21 day, 2 h daily	9.1	5.2[ab]
p-value	-	0.104	0.046
SEM	-	0.331	0.499

Different letters shows significant difference between experimental groups

The thyroid hormones level of hatched chicks is presented in Table 2. According to statistical analysis and comparison of groups mean, there is not any significant difference for T_3 concentrations between experimental groups. In groups 2 and 3, level of T_4 is significantly increased for exposed groups (groups 2 and 3), when it compared with control groups (group 1) ($p<0.05$).

DISCUSSION

Higher hatchability and lower mortality in group 5 (in comparison with other exposed groups) is according to Tarasewicz *et al.* (2006) findings that stated increase hatchability caused by exposing to low frequency MF. Increased mortality and decreased hatchability for groups 3 or 4 (exposure at second or third week) may be because of embryo sensitivity at these stages (organogenesis) to EMFs and incomplete immunological ability for environmental agents. But in group 5 may because of adaptation to EMFs from onset of incubation, hazardous effects of EMF couldn't decreases hatchability. Toman *et al.* (2002) finding as an evidence for present results suggests that intermitted or pulsative exposure to MF can increase chick embryo mortality significantly. Also, Terol and Panchon (1995) had reported that exposure to 100 Hz at last 12 days of incubation caused high mortality for quail embryo. Present findings for group 5 are according

to Tarasewicz *et al.* (2006) and for groups 3 or 4 are in agreement with Toman *et al.* (2002) and Terol and Panchon (1995) results.

Bio-electromagnetic Studies on laboratory animals stated different findings based on EMF frequency, exposure duration and physiological specify of animal (Rajkovic *et al.*, 2003; Shahryar *et al.*, 2009). In this regards, Rajkovic *et al.* (2003) had reported that exposure to 50 Hz for long time (three month) cause considerable declining in thyroid activity or T_3-T_4 levels. Also endocrine changes have been reported in pancreas of rat model in exposure to ELF-EMF (Gholampour *et al.*, 2011). Another similar study on pregnant rats shows similar elevation for thyroids hormones of exposed animals (Anselmo *et al.*, 2009). Shahryar *et al.* (2009) study with high frequency show T_4 elevation and T_3 declines follow 50 day exposure period. But only relative study (with ELF-EMF) on chicken T3-T4 status has been published by Sechman *et al.* (2006) and had completely different result with those of reported by Rajkovic *et al.* (2003) and Koyu *et al.* (2005) in mammalian model. Results of present study for T_3-T_4 hormones rate of groups 2, 3 and 5 (Table 2) that exposed at two-thirds or entire embryonic life are according to Sechman *et al.* (2006) findings. It seems that exposing to ELF-EMF couldn't cause any stress or metabolic/endocrine disorder for groups exposed only during late embryonic period (group 4) but ELF-EMF may induce endocrine changes during first third or second third of embryonic life that cause T_4 elevation. Constant rate of T_3 in experimental groups (Table 2) shows that ELF-EMF didn't has deleterious effect on basal thyroid gland activity and may affect T_3 to T_4 conversion process that present idea was suggested by Shahryar *et al.* (2009) with high frequency EMF.

CONCLUSION

In conclusion, Intermitted exposure to 50 Hz, 0.5 mT EMF during first third or second third of embryonic life can cause considerable mortality and hatchability decreases. Although exposure to EMF entire incubation period or at first third couldn't has significant effect on hatchability. Embryo mortality can be significantly higher when chick embryo submitted to EMF exposure during second third of incubation, may be because of sensitivity of embryo at organogenesis stage. Exposing to extremely low frequency of EMF during 1-14 d of incubation caused T_4 level increases, whereas T_3 level was not affected by EMF. Future completive researches for identification of possible effect of ELF-EMF on chicken embryonic thyroid gland, its activity and relative physiological aspects of discussed changes are necessary.

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