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The effect of the electric shock on embryonic development and neurophysiological traits in the chick's embryo

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Abstract. The objective of this study was to investigate the impact of stimulating the embryo during the dormancy in the incubation period. 450 eggs (Ross 308) were allocated in four treatments each three replicates. The treatments were as follow: T1 control (without shock), T2 Shocked (40) Millivolts (mV), T3 Shocked (50) (mV), T4 Shocked (75) (mV). A different voltage device was used to shock the egg, after marking the eggs with a line of iron filings to ensure electrical conductivity, eggs were shocked at different times three times a day. The results show a significant increase ($p < 0.01$) in embryonic development for embryo weight, chick body weight, Hatchability, and embryo Index (EI) for T2, T3, and especially T4. A significant increase ($p < 0.01$) in neurophysiological traits of neurons, brain weight, and Brain Index (BI) for T2, T3, and especially T4. In concluding the use of electric shock in the embryonic period will developing of the embryo and neurophysiological traits.

1. Introduction

Chick embryos like the embryos of another animal, want a lot of care as a result of it grows outside the body of the hen [1], therefore chick embryo wants a lot of biological process feeding, this can be because of the utilization of nutrients and alternative substances within the egg. The undeveloped organism goes through seizures from hibernation during an early-stage period of development and makes it incapable to utilize food, therefore, early-stage care fundamental for ideal undeveloped development.

Numerous articles recommend that the versatile activities of grown-up creatures are joined by checked changes in the cerebrum's electrical action, from the orderly transformation of biopotentials into great neuronal and cell useful modifications [2, 3]. At a similar second, information on the neurophysiological associates of versatile action in early ontogeny is not really accessible. Early formative investigations of the making of foundation electrical appearances of mind movement have just uncovered the time frames during which electrical action emerges [4, 5]. There are important roles, in early ontogeny, for main excitation rhythms detectable from indices of electrical brain activity, and which affects the lower nervous systems substantially and changes their hereditarily determined activity [6, 7].

These are motivations to accept that electrographic signals happen from the get-go in ontogeny that corresponds to the section of tangible signs into the cerebrum, and the reworking measures concerned, including the effector creation [8]. Accordingly, thus, gives a chance to expect that the unconstrained electrical action of the brain inversion a system, early ontogenic periods that arrange the variables that



direct the life form's useful cycles [9]. The issues stated focused our interest in the research of the electrical activity of the embryonic brain in direct formation, adaptive changes in engine analyzer activity during the terminal stage of embryogenesis [9]. So motivated by the idea that the embryo was stunned by an electrical stun, it can break the hibernation and accordingly help feed. The effective hatching depends on a particular incubating circumstance that is accepted by the incipient organism over 24 hours prior to incubating: the snout has gone into the air chamber and is facilitated sideways against the shell; the neck is wound; The correct side of the head confronting the airspace is covered by the traditional; Tarsal joints are secured against the shell close the point [10]. The objective of this investigation that animates of chick embryo to the utilization of substances inside the egg for getting high embryonic development and neurophysiological traits and take the best situation for successful hatching.

2. Materials and Methods

2.1. Animal Study:

The study was carried out according to the protocol approved by the University of Anbar, Ethics-Committee, Iraq. Fertile eggs from Ross (308) strain broiler breeder hens were gotten from a commercial farm.

2.2. Experimental study:

The study was carried out in the experimental farm of Animal Production Department, College of Agriculture, University of Anbar, Iraq. 450 eggs were utilized (ross 308) appropriated to five treatments every treatment 3 replicate. The research treatments were as follows: T1= control (without shock), T2= Shocked (40) Millivolts (mV), T3= Shocked (50) Millivolts (mV), T4= Shocked (75) Millivolts (mV). A different voltage device were used to shock the eggs, after marking the eggs with a line of iron filings to ensure electrical conductivity.

2.3. Studied traits:

Egg weight, Embryo Weight, Chicks Weight, and Hatchability

The egg weight for control and experimental groups ranging from 50 g to 60 g was almost the same. In 19 days of hatching, the embryo was extracted according to [12] and the embryo was weighed in 19 days of incubation. After hatching the chicks were collected from control and experimental group on the day of hatching and weighed. The percentage of hatchability was determined as (number of chicks hatched/number of fertile eggs set).

2.4. Embryo Index (EI)

All eggs in each group (control and experimental) were weighed one by one at the end of the experiment. Then they separated and cleaned their embryos. Weighed embryos in each group. The embryo index (EI) for all eggs was calculated using the formula below [13]

$$EI = \left[\frac{\text{Embryo Weight (gr)}}{\text{Egg Weight (gr)}} \right] \times 100$$

2.5. Tissue processing:

Immediately after the tissue was obtained, it was immersion fixed in 4% of paraformaldehyde at 4°C for 2 weeks. The brains were dehydrated, infiltrated and the blocks were prepared by embedding in paraplast. Serial coronal sections of 7 µm thickness were cut with a rotary microtome. The sections were mounted on egg albumin-coated glass slides and subsequently stained for Nissl substance with 1% buffered thionin. A comparison of the size of sections of the brain of experimental and control groups at a distance of 2 mm from the rostral end of the brain was done [14].

2.6. Quantification:

The measurement of the neuronal nuclear area was determined using an image analyzing system (AXIO ZEIZZ). The measurements were made under a × 100 objective lens such that pixel size was 0.51 µm [15].

2.7. Statistical Analysis

This experiment was carried by using Complete Randomized Design (C.R.D). and the Data were analyzed by using SAS program for statistical analyzing (SAS, 2001) The means for each treatment were compared by using Duncan's polynomial by using 0.05 and 0.01 significance level to determine significant differences between the averages [16].

3. Results and Discussion

Table 1 shows the electric shock on embryonic development and hatchability of chick embryos. There was a significant increase ($p < 0.01$) in embryo weight for T2, T3 and T4 (34.53, 34.93 and 35.70 g) respectively, compared with D (30.00 g). Additionally, there was a significant increase ($p < 0.01$) in body weight for T3 and T4 (41.23 and 42.23 g, respectively) compared with T1 and T2 (35.73 and 39.90 g, respectively), while T2 also had significantly increased body weight ($p < 0.01$) compared with T1. However, Table 1 shows that hatchability is significantly higher ($p < 0.01$) in T4 (77.10%) compared with other treatments T1, T2 and T3 (67.66%, 69.10% and 72.05%, respectively). T3 had significantly higher hatchability compared with T1 and T2, but there was no difference between T2 and T1.

In nature, the hen chips away at the brood of the eggs and give the embryo all the fundamental development prerequisites vital for its endurance and non-mortality this is called maternal care, one of this maternal care is to deliver a sound from the hen to the embryo as there is the vocal correspondence between the embryo and the hen, this is a sort of incitement of embryo development and improvement [17]. On the other hand, there is an expected contrast of voltage is produced from the rubbing among eggshell and plumes, the quills contain a portion of the components that produce the charge, while containing the egg on some different components, making a sort of exertion that can invigorate the embryo [18]. Extraembryonic membranes lead to egg prospective generation, Stern et al., [19] demonstrates that the potential contrast between blastoderm (negative) and albumen (positive) may achieve 8mV following 24 hours of hatching, the potential difference between embryo and albumin increased over the first four days of incubation and subsequently decay. Since the size of the embryo is only a few millimeters at this point, the shell reaching the terminal runs like an ordinary spatial shell, which radically weakens the adequacy of the surface possibilities. The quick increment of the upside negative surface potential reflects not just the difference in the outright greatness of the embryonic potential however fundamentally the development of the embryo, the weight of which rises roughly 100 times between the first and fourth day and reaches 0.1, 1.1 and 3.5 grams respectively on days 4, 7 and 10 [20]. The moderating of the dipole motion on day 5 corresponds with the sinking of the ceaselessly heavier incipient organism into the yolk. The embryo turns on its left side between days 5 and 9 and later on its back. It is gradually shifted into the big end of the egg by contractions of the amnion, while albumen gathers in the tiny end. Vince [21] demonstrate the inversion of the embryonic potential observed on days 7-10 is clearly not because of an inversion of the developing life egg white's potential inclination however to changed dispersion of the sinks and wellsprings of current inside the egg [22]. But in the artificial hatching these stimuli there aren't found, so the stimulation of embryo is necessary. However, electrical hypothalamus stimulation triggered the release of a substance through the pituitary stalk, and the portal of blood flow to the pituitary and stimulates to release the hormones are essential for growth, also neurotransmitters support growth regulatory and morphogenetic functions [23]. One of these neurotransmitters is ACh, known as acetylcholine, released from increasing axons, controls the development, differentiation and plasticity of central nervous system cells, additionally assumes a main role in the organizing of morphogenetic cell developments, cell multiplication, development, and differentiation in avian too, it plays a part in nerve impulses movement in the sympathetic system [24]. While ACh encourages the survival of spinal motoneurons in chicken that would otherwise suffer programmed cell death without trophic variables [25]. The findings acquired show that a significant reconstruction of brain electrical activity accompanies the growth of adaptive movements, which stays neutral throughout the engine analyzer's entire operating period in the new system. Assessment of EEG

rating by qualitatively distinct indices "histograms, autocorrelation and cross-correlation analyses" [4], It can be concluded that a fresh, stable motion system is developed in the context of the synchronization of the electrical mechanisms of the brain and the boost in the relative importance of changes in the dominant EEG rhythms. As evidenced by the autocorrelation assessment, the regular EEG elements are significantly amplified (with the insignificant extinction coefficient) [9]. The high voltage of electrical incitement (ES) appeared to be more reliant on myofibrillary discontinuity than on metabolic increasing speed, similarly as with low voltage. This is the reason for the increased affection with the high voltage ES [26]. While broilers have a high-voltage ES decreased at least m-calpain activity "p-calpain was completely autolysis in both ES and control muscles"[27]. That ES, on the other hand, enhanced calpain activity but had no impact on m-calpain [28]. It is a protein of the calcium-dependent, non-lysosomal cysteine proteases (proteolytic enzymes) family that are omnipresent in mammals and many other organisms, it is involved in processes such as cell mobility and the growth of cell cycles, In addition to cell-specific tasks such as long-term neuronal potency and myoblast cell fusion. At these physiological circumstances, a temporary and localized calcium flow into the cell activates a tiny local calpain population "for example, those close to Ca²⁺ channels", then progress the signal transduction path by catalyzing the proteolysis regulated by the target proteins [28]. So, the stimuli of electric according to above working in to direct during two way, the first do reduce of hibernation, makes the embryo able on the utilization of food. The second its stimuli the nervous system, thus increasing of bio-operations in the body which improved from weight of chick [29].

3.1. Physiological traits:

Figure (1) indicated to the effect of electric shock on neurophysiological traits in chick embryo, there were significantly increase ($p < 0.01$) in neurons for T2, T3 and T4 (40.34, 41.33 and 44.13 μm) consecutively, compared with T1 (35.29 μm), while T4 had significantly higher ($p < 0.01$) compared with T2 and T3, also T3 difference significantly ($p < 0.01$) than T2. However, Figure (2) demonstrated the effect of electric shock on Brain Weight, there was no difference between T3 and T4 (0.87 and 0.89 g.) consecutively, but there are significantly different ($p < 0.01$) compared with T2 and T1 (0.82 and 0.73 g.) consecutively, also there is significantly different ($p < 0.01$) for T2 compared with T1. Figure 3 illustrates the electric shock on the embryo index (EI); the EI was significantly higher ($p < 0.01$) in T2, T3 and T4 (62.19, 62.07 and 63.51, respectively) compared with D (56.76).

Electrical stimulation helps the growth and development of nerves, especially in the first stage of growth, and this will develop neurological synapse in the brain and promotes the development of nerves and thus increases the expression of the protein in the nucleus of cells [30], as well as the increased neurological synapse, leads to increased secretion neurotransmitters and hormones are essential for growth from brain, The diencephalon is said to have a nervous control of the anterior lobe, because of the facts that injury of the nucleus or fibers of the diencephalon results in atrophy or degeneration of the anterior lobe of the pituitary, that hormonal secretion from the anterior lobe increases by stimulating those nervous tracts [25]. This mechanism of electric stimulation develops during the terminal period of embryogeny and coincides in time with such essential transformations of the brain's functional properties as the establishment of stable, rhythmic electrical activity and the ability to assimilate a rhythm. The existence of these properties may be considered a sufficient criterion of the functional maturity of the brain in early ontogeny [32]. The electrical stimulation stimulates the cells to secrete glucocorticoid, which at the same time stimulate their receptors to the brain. Glucocorticoid actions in the brain are mediated by glucocorticoid receptors and mineralocorticoid receptors. Glucocorticoid receptors occur throughout the brain but are most abundant in the hippocampal, hypothalamic and pituitary area [33 and 34].

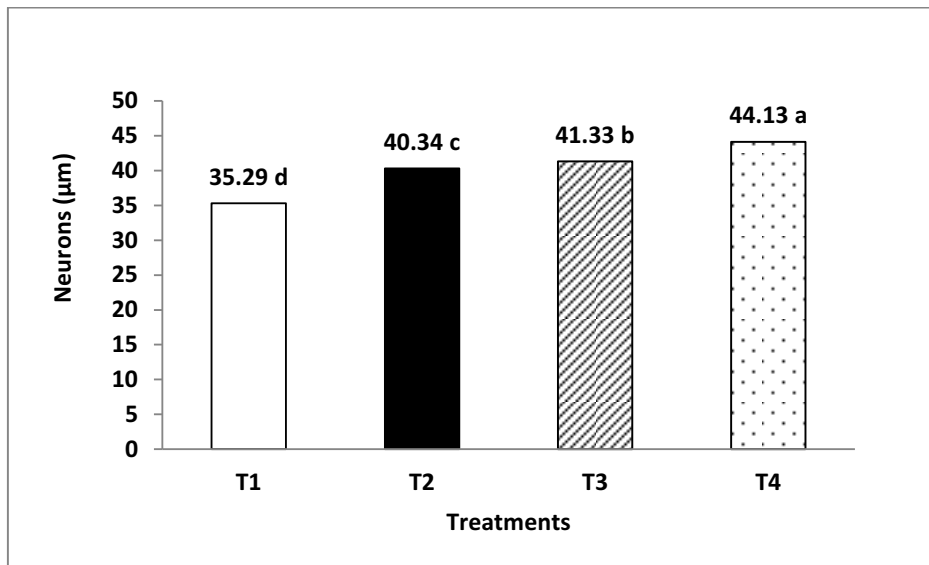


Fig. 1. The Effect of electric shock on Neurons (µm) in the chick embryo

SEM = 1.78

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.05.

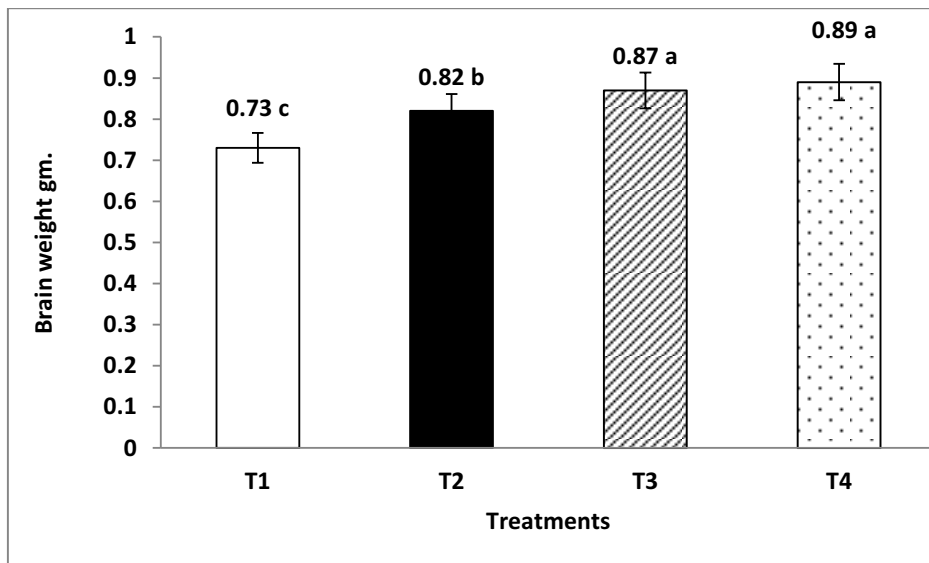


Fig. 2. The Effect of electric shock on brain weight gm. in the chick embryo

SEM = 0.1

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.05.

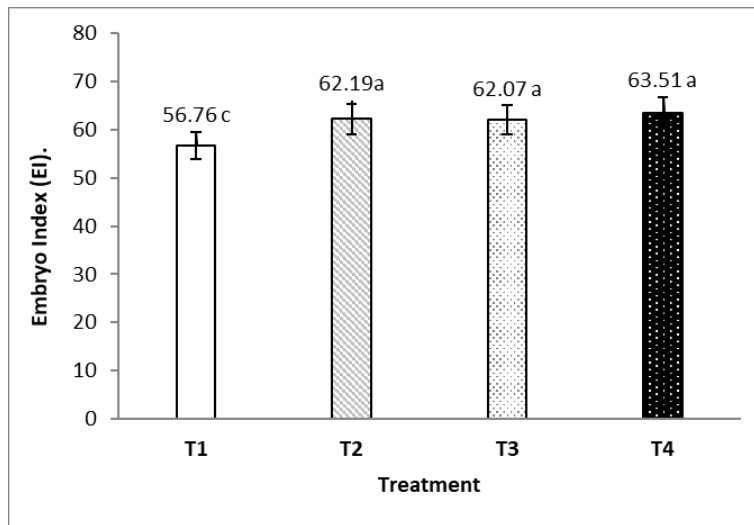


Fig.3. The Effect of electric shock on Embryo Index (EI).

SEM = 0.1

a, b, c: means in the same Rows with different superscripts differ significantly at probability value 0.05.

Table 1 shows the electric shock on embryonic development and hatchability of chick embryos.

Treatments	Egg Weight (g)	Embryo Weight* (g)	Chick Weight (g)	Hatchability (%)
T1	54.61	31.00 ^b	35.73 ^c	67.66 ^c
T2	55.52	34.53 ^a	39.90 ^b	69.10 ^c
T3	56.27	34.93 ^a	41.23 ^a	72.05 ^b
T4	56.21	35.70 ^a	42.23 ^a	77.10 ^a
Mean	55.56	33.84	39.77	71.74
**SEM	0.14	0.56	0.76	1.20
Significant	*** N.S	0.0001	0.0001	0.0001

*Embryo Weight at 19 days

** SEM: Standard Error Mean

*** N.S.: Non Significant

a, b, c: means in the same columns with different superscripts differ significantly.

4. Conclusion

In concluding that electrical stimulation works to develop embryonic growth and thus increases brain weight that helps to complete biological processes in the body, as well as adjusts physiological and neurophysiological traits to obtain the best position for successful hatching.

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