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Categprization

THE EFFECT OF THE ANTIBIOTIC GENTAMICIN ON THE STAGES OF ONTOGENESIS IN CHICKEN EGGS

Albulena BEADINI[1], Sheqibe BEADINI[1], Nexhbedin BEADINI[2], Blandi LOKAJ[3], Festina DOBRA[3], Greta KASTRATI[3], Ardit ADEMI[3], Adriana DUSHAJ[3]

[1] Department of Biochemistry, Faculty of Medical Science, University of Tetovo, Republic of North Macedonia
[2] Department of Cell and Molecular Biology, Faculty of Medical Science, University of Tetovo, Republic of North Macedonia
[3] Scientific Laboratory of Medical Science, Faculty of Medical Science, University of Tetovo, Republic of North Macedonia
[*]Corresponding author e-mail: nexhbedin.beadini@unite.edu.mk

Abstract

Introduction: Various studies have shown that the effects of the action of drugs, in addition to having positive effects, they can also provoke negative effects in different organs of the body, where by giving more doses in some cases it can exceed the therapeutic limit and become toxic and produce pathological effects in both experimental animals and humans.
Uncontrolled use and drug overdose can lead to macroscopic histopathological changes and give teratogenic, mutagenic and carcinogenic effects.
Purpose of the paper: This paper aims to study the effects of some drugs at different stages of embryonic development in chicken eggs.
The research of this paper will consist of the toxic effect of drugs and macroscopic and microscopic histopathological changes in certain stages of embryonic development of chicken eggs.
Material and method: In the study, fertilized chicken eggs were taken and incubated in the incubator for 21 days of incubation. The eggs were treated with the antibiotic gentamicin at certain stages of embryonic development. The eggs hatched on day thirteen, day fifteen, eighteen and day twentyone. Using the stereomicroscope with the macroscopic and microscopic method were analyzed ontogenetic changes in certain stages of embryonic development.
Results: The given dose of the antibiotic gentamicin can have negative effects in certain stages of embryonic development in chicken eggs.
The dose given in ampullary form in certain quantities to chicken eggs has inhibited and stagnated the processes of embryonic development at different stages compared to control eggs.
Conclusion: The given dose of gentamicin can stagnate embryonic development in certain stages and can provoke changes in certain stages of embryonic development.
This phenomenon encourages us to do even more detailed research in this direction to see the effect of the given dose and to analyze what consequences it can provoke overdoses at different stages of embryonic development and within the organism.
The lethal dose applied at certain stages of ontogenesis may have lethal consequences for the chick embryo at certain stages of embryonic development.

Keywords: dose effect, overdose of gentamicin, histopathological changes, chicken egg, teratogenesis, mutagenesis.

1. Introduction

Gentamicin is an aminoglycoside antibiotic. It exhibits bactericidal activity against aerobic gram-negative bacteria making gentamicin a good option to treat several common infections. (Chaves BJet al., 2023).Gentamicin (GM) was discovered in 1963 and was introduced into parenteral usage in 1971. Since then, gentamicin has been widely used in medicinal applications (Changhua Chenet al., 2014).
Gentamicin, like all aminoglycosides, exhibit concentration-dependent killing. Higher concentrations correlate with greater antimicrobial killing. For these reasons, clinicians should monitor peaks and troughs closely with systemic use (Chaves BJ et al., 2023).

Appropriate use of antibiotics and synthetic chemicals in chicken feed can be beneficial and results in prevention or reduction of infectious disease and increasing the efficiency and growth rate. Different chemicals, currently being used by poultry industry, are tetracycline, penicillin, chlortetracycline, oxytetracycline, nitro-furan, sulfa compounds and arsenical compounds (Bibi Nazia Murtaza et al.,2022).

- Use of gentamicin during pregnancy

Once-daily dosing of gentamicin has been extensively scrutinized in the internal medicine literature, and has been found to be at least as efficacious as traditional dosing without increasing risk of toxicity. Overwhelming evidence demonstrates that cost is significantly decreased with single-daily dosing of gentamicin. These findings have been validated in patients with postpartum endometritis and in pregnant women with choriamnionitis (Ward K et al.,2008).

- The effect of antibiotics on the hatching of chicken eggs and the growth performance of the offspring

Feeding antibiotics (bacitracin, terramycin, aureomycin, HCl, or procaine penicillin) to hens did not consistently improve hatchability of the fertile eggs. However, in one experiment there was some increase in hatchability with either bacitracin or aureomycin HCl, both in the presence and absence of supplementary vitamin B_{12} (Orville G. Bentleyet al., 1954).

High doses of the drug can lead to the developmental issues of the chicken fetus and can directly affect the turnover number of successful hatching chicks thus annual production rate of chicken. The consumption of antibiotic treated chicken, can lead to the development of antimicrobial resistance in humans.

- Embryotoxicity of gentamicin in the human embryo

Our data showed that the concentration of gentamicin in the human embryo culture medium exhibited no significant change on the development of zebrafish embryos. However, high concentration of gentamicin exposure delayed embryonic development. The survival rate significantly decreased and the malformation rate increased in a dose-dependent manner after treatment with gentamicin (Chen F et al., 2023).

In breeder farms, injecting of antibiotic into egg, result in a decrease in mortality rate. In man, following injection, gentamicin enters into the fetal circulation from the placenta (Hadi Tavakkoli et al., 2016).In this regard, neonates toxicity due to antibiotic administration is reported (Musiime GM et al., 2015). In addition, side effects such as nephrotoxicity, ototoxicity and neurotoxicity have limited the therapeutic use of antibiotics such as gentamicin (Paquette F et al., 2015), (Picard W et al., 2014). Up to now, gentamicin toxicity has been examined and described in different animal models such as rats (Quirós Y et al., 2016), rabbits (Salih Nadia, 2015), (Khaliq Tanweer et al., 2015) and humans (Bellman R, 2014), (Hadi Tavakkoli et al., 2016).

Effect of gentamicin on kidney in developing chicks.

The use of gentamicin during pregnancy is increasing owing to the following:

a) Infections by Pseudomonas aeruginosa, most enteric bacteria and Staphylococcusaureus reported by (Yoshioka H et al., 1972).

b) Maternal infections like pyelonephritis and pneumonia reported by (Weinstein AJ et al., 1976).

c) Chorioamnionitis reported by (GilstrapLC 3rd et al., 1988).

Because Gentamicin rapidly crosses fromthe placenta to the fetal circulation and amniotic fluid, knowledge about its teratogenicpotential is essential [Yoshioka H et al. (1972), Kauffman RE et al. (1975), Weinstein AJ et al. (1976), Creatsas G et al. (1980) and Gilstrap LC 3rd et al. (1988)].

In the present study, with a single therapeutic dose of gentamicin to fertilized eggs, a teratogenic effect was observed on the histological structure of kidney in the chick using light microscopy (RituSingroha et al., 2012).
The mean weight of both the right and left kidneys of the control group was greater than that of the treated (gentamicin) group, which means that there is a reduction in weight, although this difference was found statistically non-significant on applying unpaired t-test ($p>0.05$).
Effect of gentamicin on the kidney histology of chick embryos:

1. Glomerular changes
   a) Glomerulus enlargement and hypercellularity
   b) Glomerulus congestion
   c) Mesangial proliferation
2. Proximal convoluted tubules
   a) Cystic dilatation
   b) Cloudy Swelling or Hydropic transformation
   c) No change was observed in the distal convoluted tubules, the thin segments of the loop of Henle and collecting ducts in either the right or left kidneys of the treated (gentamicin) group.
3. Infiltration by inflammatory cells
4. Congestion in blood vessels (Ritu Singroha et al., 2012).

A different study showed that gentamicin can affect the organogenesis in partridge embryos at dosage of 80/kg egg-weight if injected two and three times into egg. These alterations may be due to cytotoxic and antiproliferative properties of gentamicin.
-In the brain, edema was seen. Lesions such as edema, hyperemia and thickening of the meninges were also seen in embryos.
-Myxomatous degeneration of cardiac myocytes was seen in embryos that received the highest dosage of gentamicin (80 mg/kg, three times) but in other groups, myocytes were observed normal with no sign of necrosis (HadiTavakkoli et al.,2016).
-The pathological defects observed in the liver tissue were prominent in embryos given 80 mg/kg gentamicin three times at days 4, 5 and 6 of incubation. Hepatotoxic activity of gentamicin such as swelling, congestion, vascular degeneration and necrosis have been reported by other authors (Saleemi MK et al., 2009), (Beger RD et al., 2010).
-In the kidneys, gentamicin produced histopathological alterations ranging from necrosis of the tubules and glomeruli to complete destruction and distortion of the kidneys (Hadi Tavakkoli et al., 2016).

2. Material and Methods

A total of 20 country chicken eggs (from Drenovec neighborhood, Tetovo) were weighed and incubated at 38.2 ºC, and they were divided into 6 control eggs and 54 experimental eggs.
On the 6th day of incubation the eggs were grouped into 4 groups (6 eggs Group 1; 18 eggs each other group), and then injected with antibiotics:

- Group 1: control eggs; were not injected
- Group 2: injected with 0.5ml gentamicin mixture (0.033ml gentamicin and 3ml distilled water)
- Group 3: injected with 0.5 ml gentamicin mixture (0.06ml gentamicin and 3ml distilled water)
- Group 4: injected with 0.5 ml gentamicin mixture (0.09ml gentamicin and 3ml distilled water)

On days 13-21 of incubation, the development of egg embryos from each group was studied. Embryo weight, organ formation, external size of the embryo, formation of head, beak, eyes, limbs, feathers were monitored.
The injection method used for this study was injection in the air sack of the egg.

3. Results

During the incubation of the eggs, their weight was measured at different stages of embryonic growth (**Table 1**).
Thus, normal weight loss can be observed in the first week, and then its increase in the following stages. The antibiotic injection was carried out on the 13th day. (0.5ml mixture of gentamicin with different doses)

Table 1. Determination of weight, day of hatching and status of embryonic growth of 20 chicken eggs.

Nr	Egg	Weight before incubation (gr)	Weight on day 6 of incubation (gr)	Weight on day 13 of incubation (gr)	Day of hatching/premature hatching	Status
1	1K	55.2	54.7	52.5	13	Organogenesis
2	2K	51.3	49.8	48.3	20	Successfully hatched
3	1E	51.6	49.0	48.0	13	Organogenesis
4	2E	49.6	47.8	46.4	15	Organogenesis
5	3E	70.7	67.7	64.9	18	Organogenesis
6	4E	53.5	51.7	50.2	20	Successfully hatched
7	5E	54.5	56.0	54.1	20	Successfully hatched
8	6E	51.6	49.8	48.3	20	Successfully hatched
9	7E	55.6	54.6	50.9	13	2 Blastomeres
10	8E	51.8	49.8	48.0	20	Organogenesis
11	9E	41.5	41.1	40.2	15	Organogenesis
12	10E	45.3	42.9	40.6	18	Organogenesis
13	11E	46.2	45.4	44.5	20	Organogenesis
14	12E	62.4	61.1	59.2	20	Organogenesis
15	13E	55.2	54.5	52.3	20	Blastomere
16	14E	58.5	55.4	51.7	13	Blastomere
17	15E	55.6	53.6	51.9	15	Blastomere
18	16E	55.1	54.5	52.0	18	Organogenesis

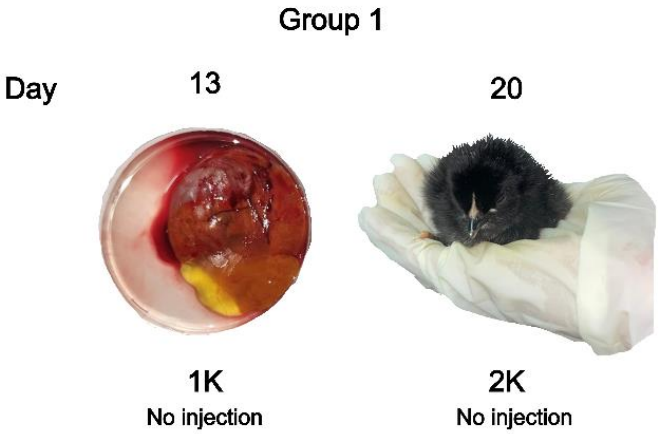
19	17E	51.7	50.0	47.9	20	Blastomere
20	18E	53.2	51.0	49.2	20	Organogenesis

On days 15-21 of incubation, the development of egg embryos from each group was studied. Embryo weight, organ formation, embryo size, formation of beak, eyes, and feathers were monitored.

Group 1 eggs hatched on different days. Thus, 1K on day 13 and 2K on day 20 (**Table 2**).

As expected, normal embryonic development is clear and distinct macroscopically.

Table 2. Embryonic development of Group 1 eggs on different monitoring days.



Experiment eggs were hatched on days 13, 15, 18, 20 to see embryonic development at different stages (**Table 3**).

On day 13, embryonic development in Group 2 is more advanced compared to Groups 3 and 4. This coincides with the lowest injection dose level.

- Egg 1E (Group 2): development in organogenesis; the development of eyes, beak, head, extremities, vascular system are clearly distinguished; the embryo breathes and is in motion, the work of the heart can be seen.
- Egg 7E (Group 3): stagnation of embryonic development was observed in 2 differentiated blastomeres
- Egg 14E (Group 4): stagnation of embryonic development in 1 blastomere.

On day 15 we get similar results, with the greatest embryonic development in Group 2, which was injected with the lowest dose of antibiotic.

- Egg 2E (Group 2): high development in organogenesis; the correct formation of the head, eyes, beak, extremities, cardiovascular system, appearance of feathers are better distinguished; the embryo breathes and is in motion, the work of the heart can be seen.
- Egg 9E (Group 3): development in organogenesis but abnormal development; the great development of the eyes can be distinguished, but the body and extremities are retarded.
- Egg 15E (Group 4): stagnation of embryonic development in 1 blastomere.

On day 18 the greatest embryonic development can be seen in Group 2, which was injected with the lowest dose of antibiotic. All 3 embryos move and breathe, which shows the development of the cardiovascular system and a high vascularization.

- Egg 3E (Group 2): highly developed embryo; macroscopically normal formation of the head, eyes, extremities, appearance of feathers and other organs.
- Egg 10E (Group 3): organogenesis, slower development; retardation in the body and extremities.

- Egg 16E (Group 4): organogenesis, development of the cardiovascular system but lag in normal development, the embryo has not grown in size, its extremities are underdeveloped, despite the development of eyes and head.

On day 20 all the remaining eggs are hatched.

- Egg 4E (Group 2): the chick is in good health; macroscopically, no deformations are seen.
- Egg 8E (Group 3): incomplete and stagnated development compared to Group 2 embryos.
- Egg 18E (Group 4): incomplete and stagnated development. Organs, head, beak, eyes are underdeveloped compared to other groups.

Table 3. Embryonic development of Group 2, 3 and 4 eggs on different monitoring days.

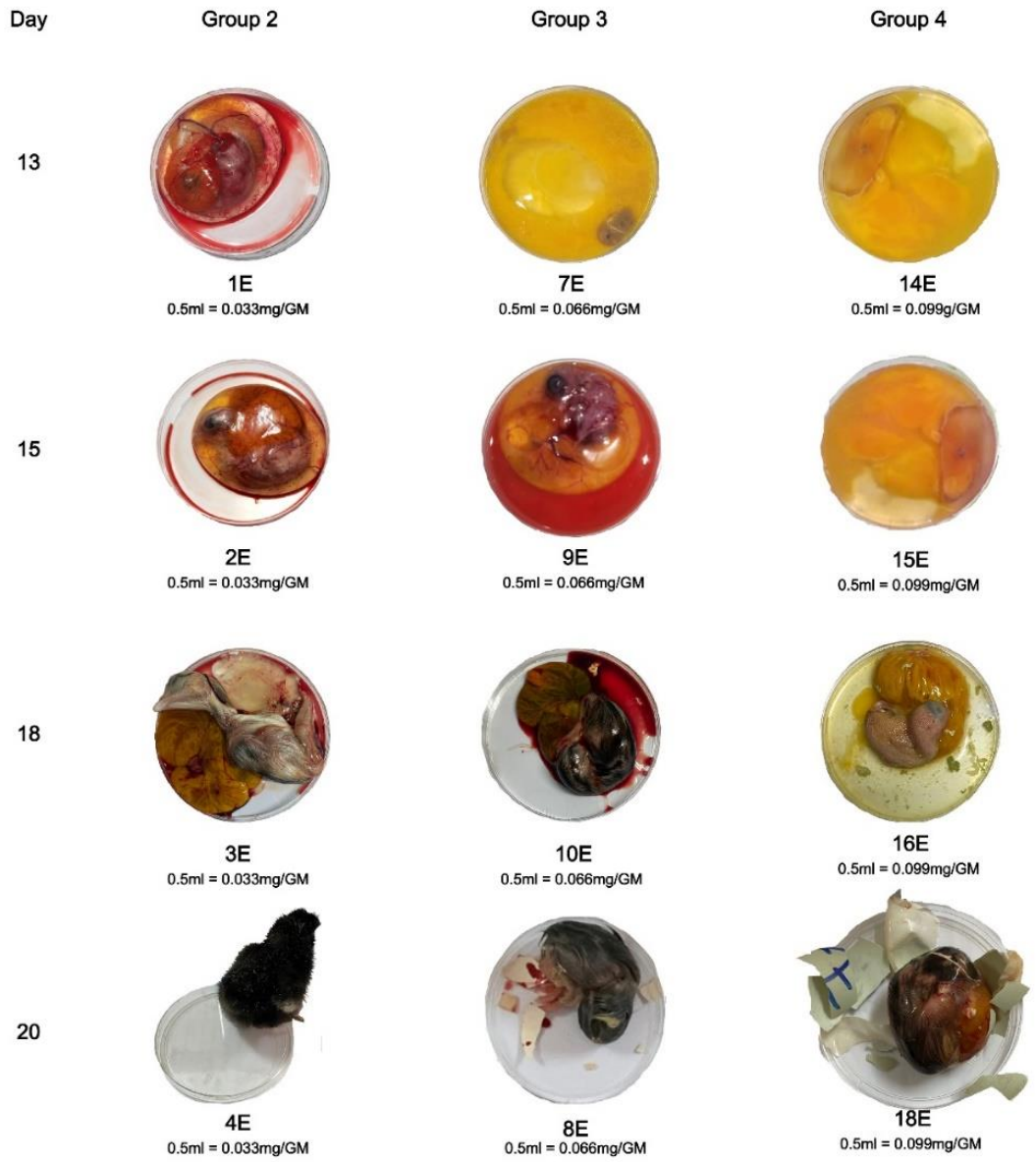


Figure 1. Group 2 healthy chicks hatched without problems.

Figure 2. Group 2 chicks in the incubator

4. Discussions

The use of gentamicin for embryo growth is not the only time research has focused on antibiotics for embryonic growth.

Use of other types of antibiotics can also be a problem. Ciprofloxacin interferes with BMP1 gene expression; it will stop mineralization of bone. This disruption will also inhibit differentiation of osteoblast results in low calcium accumulation in bone. The inappropriate administration of doses results in development of resistance to some microbes. The resistant strains can easily be transmitted to human and other animals. Hence the potential danger of presence of ciprofloxacin in meat or eggs can't be ignored (Bibi Nazia Murtaza et al., 2022).

A different study showed that gentamicin can affect the organogenesis in partridge embryos at dosage of 80/kg egg-weight if injected two and three times into egg. These alterations may be due to cytotoxic and antiproliferative properties of gentamicin (Hadi Tavakkoli et al., 2016).

Meanwhile, the use of teas for embryonic growth is also a topic worth looking into.

The given dose of 0.3 ml of mint and St. John's wort extract solution has provoked stagnation of processes during certain stages of embryonic development in quail and chicken eggs and in some cases even total inhibition of organogenesis. The given dose of 0.3 ml of the scardica and chamomile tea extract solution has resulted in normal development but also in the stimulation of processes at certain stages of embryonic development in chicken eggs and expressed differentiation in organogenesis. This phenomenon shows that not all types of teas can give positive effects at certain stages of embryonic development, therefore it is worth further researching the effect of the extract of these species because the stimulating or inhibiting effect of these teas must be proven. (Nexhbedin Beadini et al., 2023)

5. Conclusions

The results obtained from the experiment give us valuable information about the use of gentamicin in chicken eggs. In all stages when the eggs were monitored, high development was encountered only in Group 2, where the injection dose is the lowest. In other groups, development is delayed, and in some cases simply interrupted. High injection doses of the antibiotic should be avoided to prevent its harmful effects. The use of antibiotics in chicken eggs should be done with caution. The use of gentamicin for embryonic development in low doses was successful, so it can be practiced with caution. It is too early to draw a parallel with embryonic development in man. More results from more experiments are needed to reach a definitive conclusion on the use of gentamicin for embryonic development.

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