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Histopathological Evaluation of the Effect of Metronidazole on the Embryo Skin and Integument Tissues using a Chicken Embryo Model

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Abstract

Background: Metronidazole is categorized in pharmacological group C and few researches have been conducted about its pathological effects on the human fetus. Since the embryogenesis in chicken is similar to that in human beings, in the current study, the toxic effects of this drug on embryo skin and integument tissues were assessed using a chicken embryo model.

Method: The experiment was done on 36 fertilized Ross 308 eggs with the mean egg-weight of (54.4 ± 0.8g). The embryos of the control group received sterile phosphate buffered saline solution into the yolk sac on day 4 of the growing period. The embryos of the two treatment groups received metronidazole at dosages of 50 and 100 mg per Kg body-weight, respectively. The pathological effects of the drug on the embryos' skin and integument tissues were evaluated using macroscopic and histopathologic studies.

Results: According to the results, metronidazole has adverse effects on the embryo skin and integument tissues during embryonic development. Macroscopic evaluation of the organs revealed white nodules, of about 1mm in diameter, on the skin surface of the embryos. Histopathological effects of the drug consisted of hyperkeratosis, degeneration of the integument tissues and detachment from the epidermis.

Conclusion: Based on the obtained results, it is concluded that consumption of metronidazole during pregnancy can cause adverse effects on the skin and integument tissues of the human fetus. Therefore, the drug should only be given during pregnancy when benefits outweigh its risks.

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Introduction

Bacterial and protozoal infections have been always resulted in significant diseases in the medical history. Developments in the public health and different types of chemical drugs have been applied to alleviate losses in human population.

Nitroimidazole drugs have been used across the globe for many years. Today, they are used on a large scale and for different purposes. Metronidazole is an

antibiotic and antiprotozoal medication that has been commercially used since 1960 in France. Today, it is used in most areas of the world (1).

Metronidazole is primarily used to treat bacterial and protozoal vaginosis. It is used either alone or with other antibiotics to treat pelvic inflammatory disease, pseudomembranous colitis, aspiration pneumonia, rosacea, fungating wounds, intra-abdominal infections, lung abscess, periodontitis, amoebiasis, oral infections, giardiasis, trichomoniasis, and infections caused by susceptible anaerobic organisms such as *Bacteroides*, *Fusobacterium*, *Clostridium*, *Peptostreptococcus*, and *Prevotella* species. It is also often applied to eradicate *Helicobacter pylori* along with other drugs and to prevent infection in people recovering from surgery (2-6). Metronidazole inhibits nucleic acid synthesis by disrupting the DNA of microbial cells. This function only occurs when metronidazole is partially reduced and this reduction usually happens only in anaerobic cells.

Adverse effects of drugs have always been a major concern. Common adverse drug reactions associated with systemic metronidazole therapy include nausea, diarrhea, weight loss, abdominal pain, vomiting, headache, dizziness, and metallic taste in the mouth. Intravenous administration is commonly associated with thrombophlebitis. High doses and long-term systemic treatment with metronidazole are associated with the development of leucopenia, neutropenia, increased risk of peripheral neuropathy, and central nervous system toxicity. Common adverse drug reactions associated with topical metronidazole therapy include local redness, dryness and skin irritation. Metronidazole has also been associated with cancer in animal studies (7-9).

Up to now, metronidazole toxicity has been examined and described in different models such as human (10, 11), monkey (9), fish (12) mouse (13) and aquatic organisms (14). The results of these studies have showed that the possible teratogenicity and toxicity would be a determining factor minimizing potential therapeutic effect of metronidazole during pregnancy. Following administration, metronidazole crosses from placenta into the fetal circulation (15, 16). Furthermore, metronidazole is listed by the World Health Organization in the C group of the medicines. In this regard, it may produce congenital toxicity and causes adverse effects on the developing of the human fetus during pregnancy. Although increasing consumption and production of nitroimidazole drugs are predicted in some regions of the globe, little has been published about the toxic and pathological effects of these compounds on the embryonic development.

In the present study, we aimed to evaluate the histopathological effect of metronidazole on the embryonic skin and integument tissues using a chicken embryo model. A chicken embryo model was used for evaluation of the adverse effects of drug, since the embryogenesis in chicken is similar to that in human beings (17, 18). We believe that results of this study will contribute to our better understanding of safety or pathological effects of nitroimidazoles during pregnancy.

Materials and Methods

Hatching eggs

Fertile chicken eggs (Ross 308) with the average egg-weight of 54.4 ± 0.8 g were purchased from the Mahan Breeder Company, Kerman, Iran. In this company, the breeder birds were reared under the standard breeding condition.

Drugs

Metronidazole 5% injectable solution was obtained from Shahid Ghazi Pharmaceutical Company, Iran. Each milliliter of the drug contains 5 mg metronidazole. It was diluted in phosphate buffered saline solution. A volume of 0.5 mL of phosphate buffered saline solution with 50 or 100 mg metronidazole per Kg egg-weight was inoculated.

Experimental protocol

Eggs were incubated at 37.5ºC and 55% relative humidity. The eggs were randomly assigned to three equal treatment groups, 12 eggs each, as follows: Group 1: phosphate buffered saline injected group (control group) in which 0.5 ml/egg sterile phosphate buffered saline was injected to the embryonated eggs on day 4 of the incubation period. The eggs of groups 2 and 3 were, likewise, treated with metronidazole at dosages of 50 and 100 mg per Kg egg-weight, respectively. Embryos received the drug by direct injection into the yolk sac according to the standard techniques (19-22). Embryos were re-incubated after the intervention and allowed to be developed. All embryos were necropsied on day 18 of incubation and examined for the macroscopic and microscopic lesions of the skin. The experiment was performed according to the suggested European ethical guidelines for the care of animals in experimental investigations.

Pathological examination

At the end of the experiment, on day 18, embryos were humanely killed by placing on ice and then the eggs were opened at the wider end (23). After washing in normal saline solution, embryos were observed under stereomicroscope to study any gross abnormalities on the external body surface. Then, the skin section of embryos were dissected out and fixed in 10% neutral buffered formalin. Following routine preparation of tissues, serial sections of paraffin embedded tissues of 5 µm thicknesses were cut using a microtome (Slee-Germany) and stained with hemotoxylin and eosin and studied under light microscope.

Statistical analysis

Statistical analysis was performed using SPSS version 20. Chi-Squar test was used to determine significant differences in lesion occurrence between experimental groups. A P-value of <0.05 was considered as statistically significant.

Results

Macroscopic results

Macroscopic observation of the embryos in group 3, which treated with metronidazole at dosage of 100 mg

per Kg egg-weight, demonstrated lesions on the embryo's skin and integument tissues during embryonic development. The lesions revealed as white nodules, of about 1mm in diameter, on the skin surface of the embryos. In some cases, a large part of the skin was involved (figure 1). The obtained tissue samples of these embryos were sent to the pathology laboratory.

The skin and integument tissues of the embryos were normal in control group and also in the embryos of group 2, which had been treated with metronidazole at dosage of 50 mg per Kg egg-weight. In these embryos, there was not any gross abnormality in the external body surfaces (figures 2 and 3).

The viability of the embryos was checked throughout the incubation period by candling. One dead embryo was recognized in group 3, which had been received high dosage of metronidazole, at first trimester of the growing period (day 5). No mortality was observed in the embryos of the groups 2 and control.

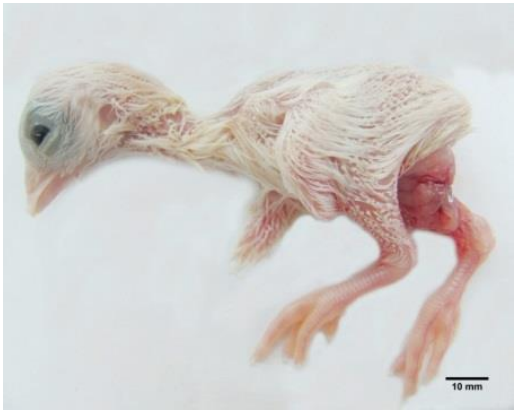

Figure 1. The chicken embryo treated with metronidazole at dosage of 100 mg per Kg egg-weight. The lesions are seen as white nodules, of about 1mm in diameter, on the skin surface.

Figure 2. The chicken embryo treated with metronidazole at dosage of 50 mg per Kg egg-weight. The embryo is normal with no gross lesion on the skin surface.

Figure 3. The chicken embryo treated with phosphate buffered saline solution. The embryo is normal with no gross lesion on the skin surface.

Microscopic results

Histopathological evaluation has been revealed that all embryos of control group as well the embryos of group 2, which had been treated with metronidazole at dosage of 50 mg per Kg egg-weight were normal. In embryos of group 3, which had been received metronidazole at dosage of 100 mg per Kg egg-weight, histopathological effects of the drug consisted of hyperkeratosis, degeneration of the integument tissues and detachment from the epidermis (figures 4-9).

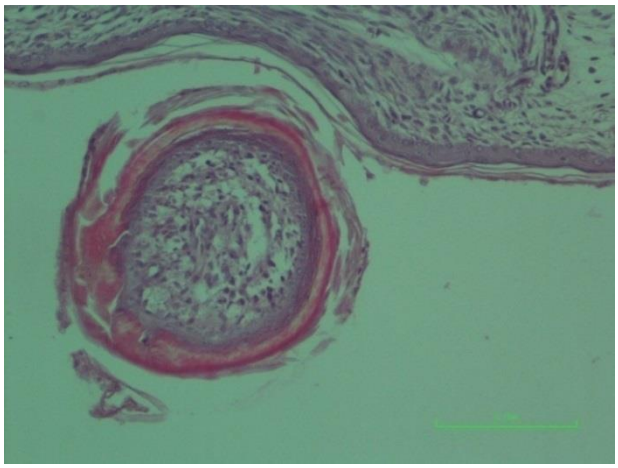

Figure 4. Photomicrograph of the chicken embryo treated with metronidazole at dosage of 100 mg per Kg egg-weight. Hyperkeratosis nodule is seen on the skin surface. ×400 H&E

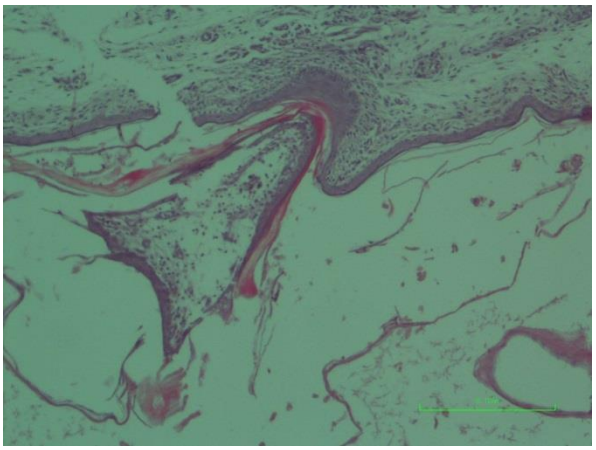

Figure 5. Photomicrograph of the chicken embryo treated with metronidazole at dosage of 100 mg per Kg egg-weight. Hyperkeratosis nodule is detached from the epidermis. ×100 H&E

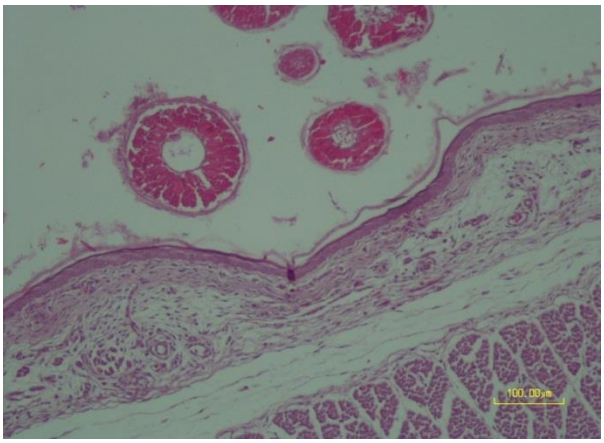

Figure 6. Photomicrograph of the chicken embryo treated with metronidazole at dosage of 100 mg per Kg egg-weight. The transverse section of the integument tissue with scattered follicles is seen. ×100 H&E

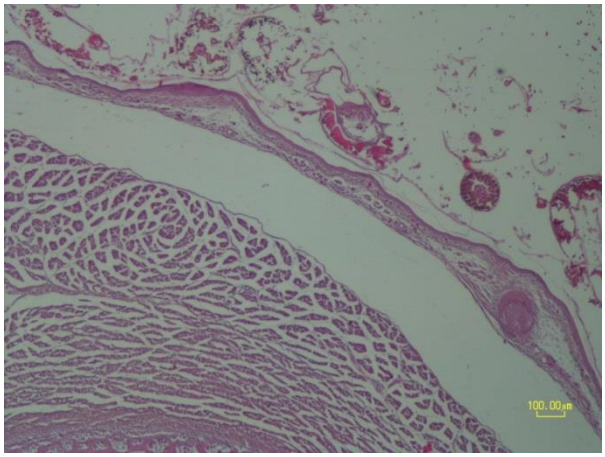

Figure 7. Photomicrograph of the chicken embryo treated with metronidazole at dosage of 100 mg per Kg egg-weight. Degeneration of the integument tissue is seen. ×40 H&E

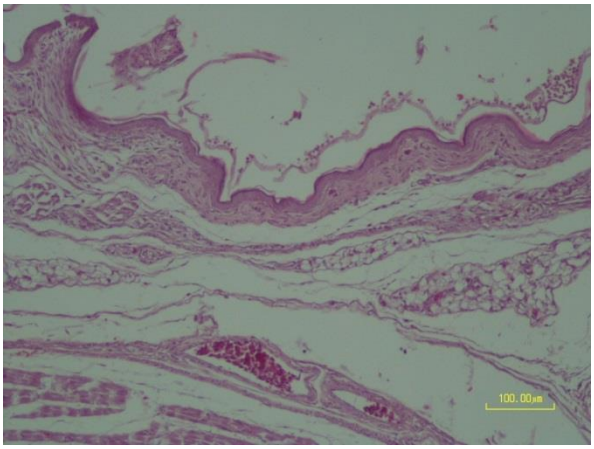

Figure 8. Photomicrograph of the chicken embryo treated with phosphate buffered saline solution (control). The normal structure of the integument tissue is seen. ×100 H&E

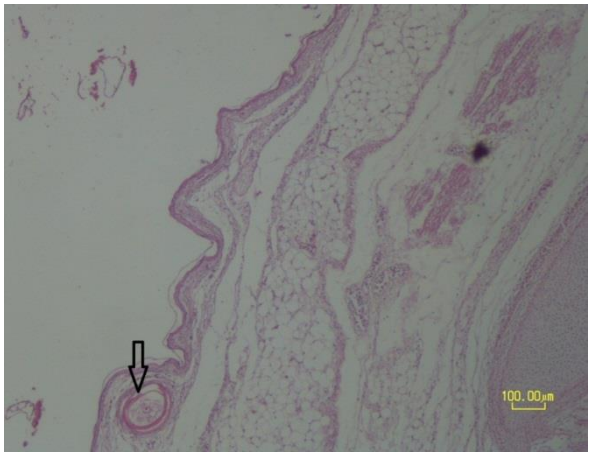

Figure 9. Photomicrograph of the chicken embryo treated with phosphate buffered saline solution (control). The normal structure of the skin with a normal follicle (arrow) is seen. ×40 H&E

Discussion

Pathogenic agents are an important and significant hazard for health and cause serious losses to the human societies. For many years, researchers have been using different drugs and chemical antibacterial compounds to restrict pathogens and enhance the public health.

There is little information available about toxicopathological effects of metronidazole on the embryonic skin and integument tissues. Therefore, in this study, we investigated cutaneous lesions following administration of metronidazole by using a chicken embryo model. Our results obviously showed macroscopic and microscopic alterations in embryos exposed to the described drug.

Metronidazole is able to cross from placenta into the fetal circulation (15, 16). On the other hand, it has been shown that the embryogenesis in chicken is similar to human beings (17, 18). It has been reported that the drug can cause adverse effect on the skin and integument tissues of the human fetus during pregnancy. Thompson et al. evaluated the early fetal exposure to vaginally-administered metronidazole in pregnant cynomolgus monkeys. Their investigation showed the presence of metronidazole and its metabolite in fetal plasma and amniotic fluid following the treatment (9). However, determining the pathological effects of the drug on human fetus requires further studies and it is suggested that the administration of metronidazole should be with caution during pregnancy.

There are few studies in the literature describing the histopathological effects of metronidazole on the fetal cutaneous and integument tissue. Hypersensitivity reactions, such as rash and itch, are associated with metronidazole administration in human (24, 25). In some avian species, skin lesions have been seen due to the metronidazole treatment (26). In addition to skin lesions, metronidazole is also able to cause specific neuro-teratogenic lesions during pregnancy. For example, the possible association between vaginal treatment with metronidazole and congenital hydrocephalus has been described previously (11). In mammals' fetus, some teratogenic effects have also been noticed after the administration of metronidazole. Axial skeletal defects in mouse fetuses subjected to the in-utero exposure to metronidazole have been reported (13). Pathological effects of metronidazole on fertility and testicular function in male rats, including infertility, inhibition of spermatogenesis, degenerative fragmentation of spermatozoa and spermatids and severe degeneration of the seminiferous epithelium, have also been described (27). Kołodziejska et al. investigated the aquatic toxicity of metronidazole using tests with marine bacteria (Vibrio fischeri), green algae (Scenedesmus vacuolatus), duckweed (Lemna minor) and crustaceans (Daphnia magna). Their data showed the strong influence of this drug on aquatic organisms and contributed to a sound assessment of the environmental hazards posed by commonly used nitroimidazoles (14).

Mechanisms involved in nitroimidazole-induced lesions have been assessed in various studies. These mechanisms are summarized as follows:

A) Free-Radical Intermediates: The genotoxic action of nitro compounds requires the enzymatic reduction of

the nitro group. The reduction scheme includes free radical intermediates. There are three potential free-radical intermediates of enzymatic nitro reduction: the nitro anion ($R\text{-}NO_2$-), the hydronitroxide (R-HNO), and the amino cation free radical ($R\text{-}NH_2$+).These free radicals could be of importance in the toxicity of nitroimidazole compounds (28, 29).

B) Covalent binding of the free radical metabolite to tissue macromolecules including proteins and DNA (29).

C) The oxygen-reduction byproducts, such as O_2-, H_2O_2 and OH-, generated by the autoxidation of the nitro anion radicals. The oxidation of nitro group and anion free radicals is the dominant pathway in the presence of O_2-. This reaction results in regeneration of the nitro compound and production of O_2- , that its dismutation yields H_20_2 and OH- (30, 31).

D) The electron affinity (redox potential) of the nitroimidazoles (32).

E) Hypoxic cell toxicity. One of the major concerns in the clinical application of nitroimidazoles is that they may be cytotoxic to the normal hypoxic tissues (nerve, skin, cartilage, etc). For instance, side effects such as skin eruptions, polyneuropathy, and psychic disturbances are usually observed after treatment with nitroimidazoles (32, 33).

Our study obviously showed histopathological lesions of metronidazole on the fetal cutaneous and integument tissue. The mechanisms of the lesions have not been exactly defined and further studies are required to elucidate the underlying mechanisms.

Conclusion

This experimental study on the chicken embryo model revealed that metronidazole causes adverse effects on the embryo skin and integument tissues during the embryonic development. Based on the results obtained from this model, it is concluded that consumption of metronidazole during pregnancy can cause cutaneous lesions in human fetus. Therefore, It is suggested that the drug be used with caution during pregnancy or only be given when benefits outweigh its risk.

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References

1. Pasupuleti V, Escobedo AA, Deshpande A, Thota P, Roman Y, Hernandez AV. Efficacy of 5-Nitroimidazoles for the Treatment of Giardiasis: A Systematic Review of Randomized Controlled Trials. *PLoS Negl Trop Dis* 2014; 8(3):2733 -9.
2. Mombelli A, Almaghlouth A, Cionca N, Courvoisier DS, Giannopoulou C. Differential benefits of amoxicillin–metronidazole in different phases of periodontal therapy in a randomized controlled crossover clinical trial. *J Periodontol* 2015; 86(3):367-75.
3. Nishizawa T, Maekawa T, Watanabe N, Harada N, Hosoda Y, Yoshinaga M, et al. Clarithromycin versus metronidazole as first-line Helicobacter

pylori eradication: a multicenter, prospective, randomized controlled study in Japan. *J Clin Gastroenterol* 2015; 49(6):468-71.

4. Taieb A, Ortonne J, Ruzicka T, Roszkiewicz J, Berth-Jones J, Peirone M, et al. Superiority of ivermectin 1% cream over metronidazole 0· 75% cream in treating inflammatory lesions of rosacea: a randomized, investigator blinded trial. *Br J Dermatol* 2015; 172(4):1103-10.
5. Mazuski JE, Gasink LB, Armstrong J, Broadhurst H, Stone GG, Rank D, et al. Efficacy and Safety of Ceftazidime-Avibactam Plus Metronidazole Versus Meropenem in the Treatment of Complicated Intra-Abdominal Infection–Results from a Randomized, Controlled, Double-Blind, Phase 3 Program. *Clin Infect Dis* 2016; 62(11):1380-9.
6. Williams C, Vacca A, Dunham L, Lloyd D, Coogan MP, Evans G, et al. The redox-active drug metronidazole and thiol-depleting garlic compounds act synergistically in the protist parasite Spironucleus vortens. *Mol Biochem Parasitol* 2016; 206(1):20-8.
7. Hobbs K, Stern-Nezer S, Buckwalter MS, Fischbein N, Caulfield AF. Metronidazole-Induced Encephalopathy: Not Always a Reversible Situation. *Neurocrit Care* 2015; 22(3):429-36.
8. Wu N, Nguyen J, Ahmed A. A Case Report of Metronidazole-Induced Central Nervous System Complications (P4. 396). *Neurology* 2016; 86(16):396-401.
9. Thompson KE, Newcomb DL, Moffat GJ, Zalikowski J, Chellman GJ, McNerney ME. Evaluation of early fetal exposure to vaginally-administered metronidazole in pregnant cynomolgus monkeys. *Reprod Toxicol* 2016; 59:17-21.
10. Karamanakos P. The possibility of serotonin syndrome brought about by the use of metronidazole. *Minerva Anestesiol* 2008; 74(11):679-83.
11. Kazy Z, Puho E, Czeizel AE. Teratogenic potential of vaginal metronidazole treatment during pregnancy. *Eur J Obstet Gynecol Reprod Biol* 2005; 123(2):174-8.
12. Gürcü B, Koca YB, Özkut M, Tuğlu Mİ. Matrix changes due to the toxic effects of metronidazole in intestinal tissue of fish (Onchorhynchus mykiss). *Chemosphere* 2016; 144:1605-10.
13. Tiboni GM, Marotta F, Castigliego AP. Teratogenic effects in mouse fetuses subjected to the concurrent in utero exposure to miconazole and metronidazole. *Reprod Toxicol* 2008; 26(3-4):254-61.
14. Kołodziejska M, Maszkowska J, Białk-Bielińska A, Steudte S, Kumirska J, Stepnowski P, et al. Aquatic toxicity of four veterinary drugs commonly applied in fish farming and animal husbandry. *Chemosphere* 2013; 92(9):1253-9.
15. Heisterberg L. Placental transfer of metronidazole in the first trimester of pregnancy. *J Perinat Med* 1984; 12(1):43-5.
16. Sweetman SC, Pharm B, PharmS F. Martindale: The Complete Drug Reference. 36 ed, London, Pharmaceutical Press, 2009.
17. Männer J, Seidl W, Heinicke F, Hesse H. Teratogenic effects of suramin on the chick embryo. *Anat Embryol (Berl)* 2003; 206(3):229-37.
18. Singroha R, Srivastava S, Chhikara P. Effect of Gentamicin on kidney in developing chicks. *Eur J Anat* 2012; 16(2):119-26.
19. Cheled-Shoval S, Amit-Romach E, Barbakov M, Uni Z. The effect of in ovo administration of

mannan oligosaccharide on small intestine development during the pre-and posthatch periods in chickens. *Poult Sci* 2011; 90(10):2301-10.

20. Li X-G, Sui W-G, Yan H-C, Jiang Q-Y, Wang X-Q. The in ovo administration of L-trans pyrrolidine-2, 4-dicarboxylic acid regulates small intestinal growth in chicks. *Animal* 2014; 8(10):1677-83.
21. Liu H, Wang J, Chen X, Zhang R, Yu H, Jin H, et al. In ovo administration of rhIGF-1 to duck eggs affects the expression of myogenic transcription factors and muscle mass during late embryo development. *J Appl Physiol* 2011; 111(6):1789-97.
22. Kulesa PM, McKinney MC, McLennan R. Developmental imaging: the avian embryo hatches to the challenge. *Birth Defects Research Part C: Embryo Today: Reviews* 2013; 99(2):121-33.
23. Jacobsen ID, Grosse K, Hube B. Embryonated chicken eggs as alternative infection model for pathogenic fungi. *Methods Mol Biol* 2012; 845:487-96.
24. García-Rubio I, Martínez-Cócera C, Magadán SS, Rodríguez-Jiménez B, Vázquez-Cortés S. Hypersensitivity reactions to metronidazole. *Allergol Immunopathol (Madr)* 2006; 34(2):70-2.
25. Reich A, Stander S, Szepietowski JC. Drug-induced pruritus: a review. *Acta Derm Venereol* 2009; 89(3):236-44.
26. Riviere JE, Papich MG. Veterinary pharmacology and therapeutics, John Wiley & Sons, 2013.
27. McClain RM, Downing JC, Edgcomb JE. Effect of metronidazole on fertility and testicular function in male rats. *Fundam Appl Toxicol* 1989; 12(3):386-96.
28. Asín-Prieto E, Soraluce A, Trocóniz IF, Cimarras EC, de Ugarte Sobrón JS, Rodríguez-Gascón A, et al. Population pharmacokinetic models for cefuroxime and metronidazole used in combination as prophylactic agents in colorectal surgery: Model-based evaluation of standard dosing regimens. *Int J Antimicrob Agents* 2015; 45(5):504-11.
29. Workman P, Twentyman PR, Lee FY, Walton MI. Drug metabolism and chemosensitization. Nitroimidazoles as inhibitors of drug metabolism. *Biochem Pharmacol* 1983; 32(5):857-64.
30. Duchêne M. Metronidazole and the Redox Biochemistry of Entamoeba histolytica. Amebiasis, Springer, 2015. pp. 523-41.
31. Khan AAP, Khan A, Asiri AM, Azum N, Rub MA, Khan SB, et al. Study of the base-catalysed oxidation of the anti-bacterial and anti-protozoal agent metronidazole by permanganate ion in alkaline medium. *Res Chemic Intermed* 2014; 40(4):1703-14.
32. Adams G, Flockhart I, Smithen C, Stratford I, Wardman P, Watts M. Electron-Affinic Sensitization VII. A Correlation between Structures, One-Electron Reduction Potentials, and Efficiencies of Nitroimidazoles as Hypoxic Cell Radiosensitizers. *Radiat Res* 2012; 178(2):183-9.
33. Hong CR, Wang J, Hicks KO, Hay MP. Efficient Protocol for the Identification of Hypoxic Cell Radiosensitisers. Tumor Microenvironment, Springer, 2016. pp. 269-90.