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Study of the effect of caffeine on the local formation of the fetus

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دراسة تأثير الكافيين على التكوين الجنيني في الدجاج المحلي

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Abstract:
The stage of embryonic formation in chickens is divided into two parts, and these divisions are helped by the mother's body temperature through the mother herself incubating the eggs or in the hatchery. The stage of embryonic formation is divided into 3 stages as follows: The first stage lasts from 1-4 days and is called the stage of formation of internal organs. The second stage from 5 - 14 days, which is called the stage of formation of external organs, and the third stage of increase in size, which is from 15-20 days, and is called the growth stage.
Caffeine is a commonly used and widespread substance and is one of the most consumed dietary components. It is found naturally in the leaves, seeds and fruits of more than 63 plant species around the world, and is also found in common beverages including coffee, tea and soft drinks.
In this research, the effect of caffeine on embryogenesis in local chickens was studied. 90 eggs were collected and divided into three groups. 30 eggs each week were divided into 10 control samples, 10 were injected with a concentration of 1 mM, and 10 were injected with a concentration of 5 mmm. The results showed that excessive concentration of caffeine has harmful effects and deformities, including delayed growth and death of fetuses.
It has been shown that the appropriate concentration must be taken into account to obtain the optimal result. When the concentration of caffeine increases significantly, it gives negative results and may lead to deformities. Therefore, studies and experiments must be intensified and knowledge of the use of caffeine concentrations is required because of its effects in any field. life's different aspects.

Keywords: Eggs, Growth Stage, Caffeine, Fetus, Chicken.

الملخص
تنقسم مرحلة التشكل الجنيني في الدجاج إلى قسمين ويساعد على حدوث هذه الانقسامات درجة حرارة جسم الأم بواسطة احتضان الأم نفسها للبيض أو في الفقاسة وتنقسم مرحلة التشكل الجنيني إلى 3 مراحل كالتالي :المرحلة الأولى من 1-4 أيام وتسمى بمرحلة تكوين الأعضاء الداخلية المرحلة الثانية من 5-14 يوم وتسمى بمرحلة تكوين الأعضاء الخارجية والمرحلة الثالثة الزيادة في الحجم وهي من 15-20 يوم وتسمى بمرحلة النمو.
الكافيين هو عبارة مادة تستعمل بشكل شائع وواسع الانتشار وهو واحد من أكثر المكونات الغذائية استهلاك حيث يتواجد بشكل طبيعي في أوراق وبذور وثمار أكثر من 63 صنف نبات يحول العالم، حيث يتواجد أيضا في المشروبات الشائعة بما في ذلك القهوة والشاي والمشروبات الغازية.
وتم في هذا البحث دراسة تأثير الكافيين على التكوين الجنيني في الدجاج المحلي حيث جمع 90 بيضة تم تقسيمها إلى ثلاث مجموعات 30 بيضة كل أسبوع قسمت 10 عينات ضابطة، 10 تم حقنهم بتركيز1 ملي مولر، 10 تم حقنهم بتركيز 5ملي مولر، وقد أظهرت النتائج أن الإفراط في زيادة تركيز مادة الكافيين يكون له أثار ضارة وتشوهات تمثلت في وتأخر نمو الأجنة وموتها.
وقد تبين أنه يجب مراعاة التركيز المناسب للحصول على النتيجة الأمثل، فعند زيادة تركيز مادة الكافيين بشكل كبير، فإنه يعطي نتائج سلبية وقد يؤدي إلى تشوهات، وعليه فإنه يجب التكثيف من الدراسات والتجارب والاطلاع على استخدام تراكيز مادة الكافيين لما لها من تأثيرات في أي مجال من مجالات الحياة المختلفة.

Introduction

Embryogenesis in chickens begins in the cell consisting of the union of the sperm nucleus and the egg nucleus in a process known as fertilization. After the fertilization process, the fertilized egg is formed, or what is known as the zygote. The fertilized egg begins to go through the stages of mitosis until 2, 4, 8, 16, and 32. The female lays the fertilized egg. In a suitable place for incubation, when the eggs are incubated, embryonic growth begins, and at the end of incubation, the embryo grows, occupying the internal space of the egg, after the end of its incubation period, it punctures the egg to hatch from it and be able to exit [2].

Congenital malformations are used to describe the structural, behavioural, functional and metabolic disorders present in the fetus. The abnormal structure that includes part of the body's structures is called an anomaly. Such small anomalies do not cause harm to the health of the individual but are even associated in certain cases with major abnormalities. The anomaly that constitutes most of the organic structure in the fetus is called a deformed fetus (Monster). In these cases, it may result in a complete or partial absence of a certain structure or a change in the natural form of that structure. Also, if the deformities are large, it leads to the death of the fetus [1].

Chicken eggs, specifically their embryos, are very commonly used in developmental biology studies because of their simplicity and similarity to human embryos. They are also economically efficient and can be easily manipulated in the laboratory. In addition, chicken eggs are also widely used in the study of embryonic deformities of drugs and chemicals such as caffeine [17]. Experiments were conducted on chicken embryos to test the effects of caffeine, concluding that toxicity caused by caffeine can lead to fetal deformities and even death of the fetus [11].

Caffeine is an alkaloid, (from the methylxanthine family). In its natural state, it is found in the form of a white powder with a bitter taste. It is commonly and widely used. It is one of the most consumed food ingredients, as it is found naturally in the leaves, seeds, and fruits of more than 63%. A plant species around the world, it is also found in common beverages including coffee, tea, soft drinks, as well as chocolate and a variety of medicines. Today, approximately 80% of the world's population consumes products containing caffeine daily. Caffeine is used again because it acts as a stimulant for the nervous system and is used as a stimulant for the nervous system. It also to reduce physical stress, as it prevents drowsiness and helps wake up. Studies have shown that caffeine has harmful effects, especially for children and pregnant women, represented by fetal deformities and affects the blood vessels and heart, as well as the nervous system, the cornea of the eye, and the placenta [7].

Many studies and research have been conducted around the world for the purpose of identifying the effect of caffeine on fetuses. [14] A study was conducted under the title of the toxic effects of caffeine on chicken embryos, where the results indicate that caffeine causes defects in the neural tube through its inhibitory effect on the contractile activity of the oral microcapillary bundles in developing neural epithelial cells. [18] A study was conducted entitled High doses of caffeine given to rats, where they found that caffeine leads to improper growth of many organs, causing, for example, deformities of the limbs, defects in the skeleton, cleft palate, and also hematomas, as the limbs were affected. The left one is more than the right one. [9] A study was conducted on the effect of caffeine on the heart. It was found that caffeine has a sharp effect on the heart and blood vessels and works to increase the contraction of the heart muscle and dilate the blood vessels in the fetus. [5] There is a study entitled the effect of caffeine intake on chicken embryos. The effect of caffeine on heart formation showed that it produces complete cardiac malformations in the fetus. The main malformations included pulmonary aortic trunk and aortic hypertrophy accompanied by a ventricular septal defect without pulmonary stenosis, within 3 minutes after caffeine exposure, common ventricular dilation and weak ventricular contractility were observed and persisted for 1 hour. [6] A study was conducted on caffeine consumption by pregnant women. It was found that there were different results regarding negative outcomes for the fetus, represented by the risk of spontaneous miscarriage and delayed fetal growth. [8] Researchers studied the effect of high doses of caffeine on calcium loss and its effect on normal bone growth in mice, where they found that it caused more serious negative effects on bones, including a 70% loss of bone minerals, and a decrease in mineral density. Bones and low calcium content.

[15] There is a study of the negative effect of caffeine on bone metabolism, which found that drinking coffee causes the risk of osteoporosis and is characterized by a decrease in bone mineral density and an increased susceptibility to fractures. [4] A study was conducted on the effect of caffeine on fetal death, where it was found that there is a high risk of fetal death according to coffee consumption during pregnancy, and the risk increases with the increase in the number of cups of coffee per day. [10] conducted a study on caffeine toxicity in humans in case of taking an overdose of caffeine, as it affects a variety of receptors and has profound side effects as it can lead to irregular heartbeat, tachycardia, vomiting, and convulsions. And coma. [20] studied the effect of high doses of caffeine intake during

pregnancy, with results indicating that it increases the risk of miscarriage, regardless of pregnancy-related symptoms. [19] A study was conducted on fetal exposure to caffeine in rats where it was found to lead to adverse effects in adulthood as it can have acute effects on the fetus and long-term effects in adulthood. [12] Researchers studied the effect of caffeine on chicken embryos during the embryonic period, where changes were observed in all layers and the total thickness of the cornea was reduced. [13] A study was conducted on the interference of caffeine in embryonic development through excessive stimulation of serotonergic hormone in the chicken embryo, where it was found that abnormal closure of neural tubes in caffeine clusters during the early stage causes a disturbance in the development of the serotonergic system, which may It increases the rate of fetal malformation, and at the same time is likely to be a major factor in causing psychological and behavioral disorders in adults.
[16]. A study was conducted on the possible effects of caffeine on chicken embryos, the results of which recorded a very high mortality rate, and the results of the surviving embryos showed deformities that included anencephaly, microcephaly, and a delayed nervous system.

Factors that lead to birth defects:
1- Genetic factors: One of the most important conditions that cause congenital malformations due to genetic factors is what is known as Down or Darwin syndrome (excess chromosome number 21).
2- External environmental factors: They include radiation, viruses, medications, chemicals, antibiotics, hormones, and diseases [2].
The embryonic stages in which the influence of external factors varies:
1- The pre-differentiation stage: When the embryos at this stage are exposed to an external factor, some of the embryo's cells are damaged and the embryos die. When some of the embryo's cells remain alive without being damaged, they compensate for what was damaged and continue their formative journey.
2- The critical or sensitive stage: This is the stage of organ formation and establishment. If the fetus at this age is exposed to any external factor (medications, radiation), some organs will soon be affected and change from their normal path to give an abnormal and distorted appearance.
3- The growth stage: In this stage, the organs grow and when they are exposed to one of the previous factors, they can cause some minor deformities to the formed embryos, and the effect is greater on the physiological aspects of the embryos. This is because the sensitivity of the fetal cells to the deforming factor gradually decreases as the fetus grows older [2].
Caffeine: Caffeine is an alkaloid (from the methylxanthine family). In its natural state, it exists in the form of a white powder with a bitter taste and is commonly and widely used.
The scientific name:
(1,37, Trimethyl Xanthine)
Molecular formula:
C8H10N4O2
The effect of caffeine on fetuses
1- Heart defects.
2- Structural defects.
3- Delayed fetal growth.
4- Closure of the neural tube.
The difference in deformation cases: is due to several factors:
1- Caffeine intake
2- Dosage and exposure time.
Chicken classification:
Kingdom: Animalia
Phylum: chordate
Subphylum: vertebrata
Class: Ave
Order: Galliformes
Family: Phasianidae
Genus: Gallus
Species: Gallus gallus [3].
The aim of the study:
The study aims to determine the extent of the effect of caffeine on embryogenesis in local chickens at different concentrations and to compare injected embryos with non-injected embryos during the embryogenesis period in the first, second and third weeks.

Materials and working method:
Materials:
Study samples: In this study, 90 fertilized eggs were collected from a chicken barn in the Iqzir area in the city of Misrata. The collection period took about two weeks (2 months), and then 90 eggs were divided into three groups. The first group contained 30 eggs that were not injected (control samples) (A) and the group The second group (B) contains 30 eggs that were injected with a concentration of (1Mm), and the third group (C) contains 30 eggs that were injected with (5mM), during the study period of 21 days.
Equipment and materials used: The following materials were used in this study
•Hatchery eggs.
• Sensitive balance.
• Hot plate heating device.
• Paraffin wax.
• Needles for injection.
• Test tubes.
• Distilled water.
•alcohol.
•gloves.
• Petri dishes.
• Caffeine from Sigma-Andrich, Lewis, USA.

Material and methods
Preparation of caffeine concentrates:
1- The caffeine concentration (1mM) was prepared: 1.94 milligrams of caffeine was taken with 10 milliliters of distilled water and placed in a test tube with continuous shaking.
2- The caffeine concentration (5mM) was prepared: It was prepared by weighing 9.7 milligrams of caffeine with 10 milliliters of distilled water and placing it in a test tube with continuous shaking.
The process of injecting eggs and sealing them with paraffin wax:
1. The injection site was sterilized with alcohol.
2. The egg shell was pierced with an injection needle in the non-vibrating part, and then we injected it with a concentration of 1 mm of caffeine.
3. The paraffin wax was heated using a heating device.
4. We close the hole with paraffin wax, then put the eggs in the incubator at a temperature of 37°C.
The fertilized eggs were placed in a hatchery during the study period, which lasted 21 days. Samples were monitored daily and examined at home to compare the injected and non-injected eggs. 10 eggs from the first group injected with caffeine at a concentration of 1 mm were examined, 10 from the eggs injected with caffeine at a concentration of 5 mm from the second group, and 10 From the non-injected eggs (control samples), the eggs were hatched in Petri dishes to identify the embryonic composition and the differences between the injected and non-injected embryos, compare the non-injected and caffeine-injected samples, and observe the extent of deformities after 7 days, then after 14 days, and then after 21 days.

statistical analysis:
We used the SPSS program to conduct statistical analysis to study the difference between the effect of caffeine on embryos injected with caffeine and embryos not injected, in the three groups of local chickens, using the analysis of variance (ANOVA) test.

Results & Discussion Results & discussion:
Sample examination results:
90 samples of locally fertilized eggs were examined during different stages of this study, identifying differences between embryos injected with caffeine and embryos not injected with caffeine, and recording observations at the following periods:
Embryos after 7 days: Embryos not injected with caffeine. The spine and head began to form, the eyes appeared, the front and back limbs appeared intact, and the blood vessels were clear, as in Figure

(1-A). As for the embryos injected with caffeine at a concentration of 1 mm, we notice the occurrence of deformities represented by the yolk with the white, as in Figure (1-B), and in embryos injected with 5 mm caffeine, we notice an increase in the concentration of caffeine, which led to increased mixing of the yolk with the white, and a delay in growth, as in Figure (1-C).

1(a) 1(b) 1(c)

Figure 1 Embryos after 7 days (A - Embryos not injected with caffeine, B - Embryos injected with 1Mm of caffeine, C - Embryos injected with 5mM of caffeine).

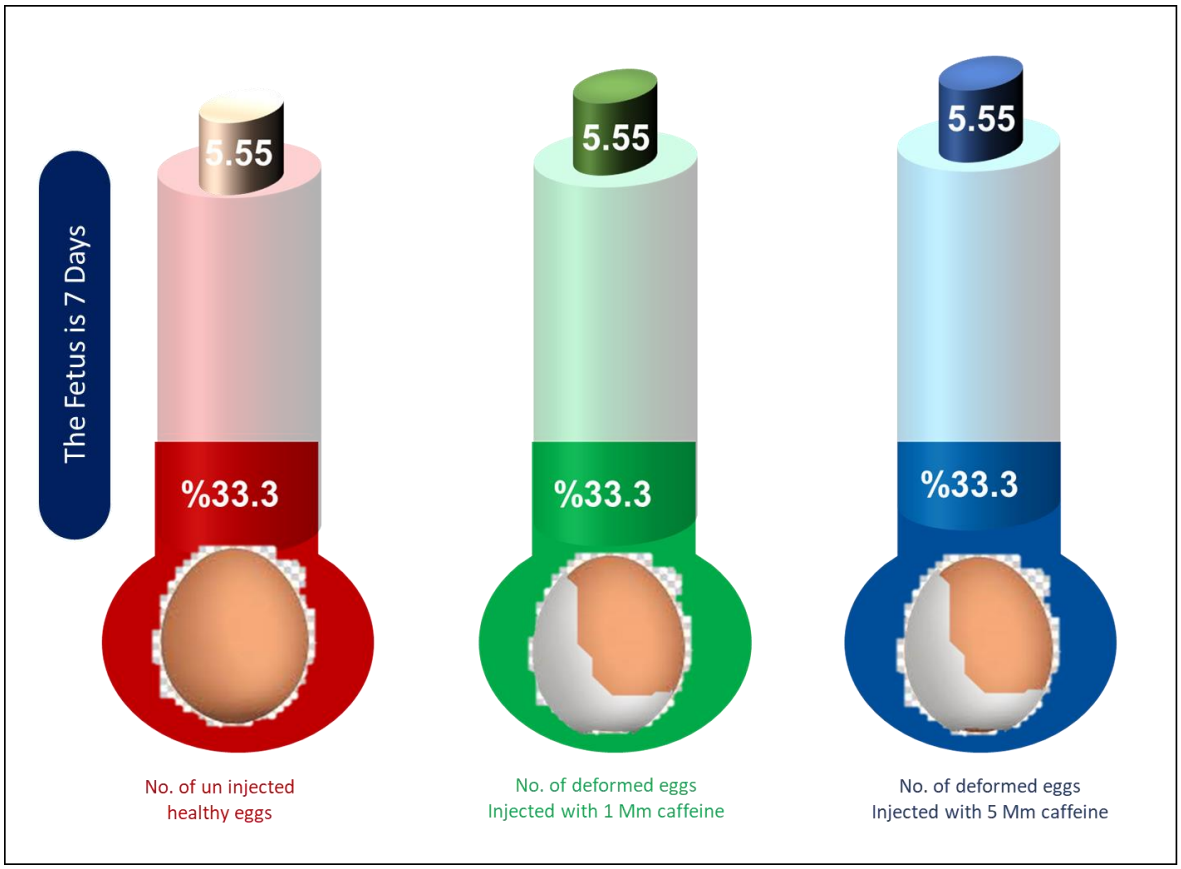
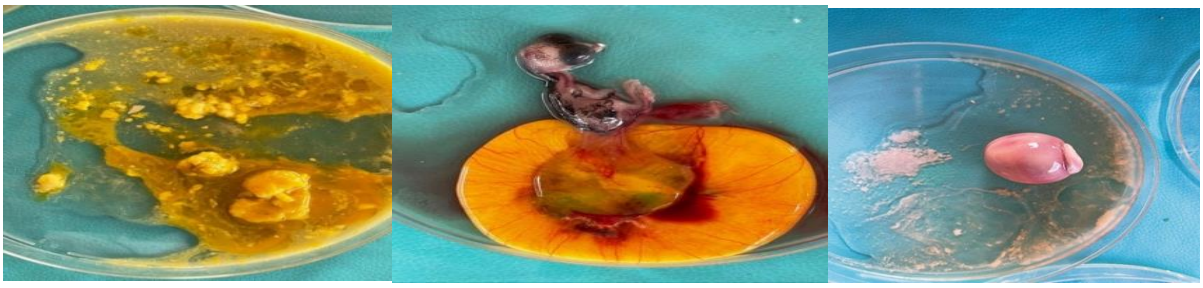


Figure 2 The difference in the rate of deformities between the group of native eggs not injected with caffeine, the deformed eggs injected with 1 mM caffeine, and the deformed eggs injected with 5 mM caffeine at the age of 7 days.

We note that in the control samples, the size of the embryo and the blood vessels are normal, compared to the deformed eggs injected with 1mM. Deformities occurred in the form of mixing of the yolk with the white and in the deformed eggs injected with 5mM, an increase in the concentration of caffeine led to increased mixing of the yolk with the white and a delay in growth.

Embryos after 14 days: It was observed in the embryos not injected with caffeine (control samples) that the fore and hind limbs appeared normally, the beak formed, an increase in the size of the eye, the appearance of fluff, the appearance of claws, and the embryo surrounded by the yolk area, as in Figure (3-A). We also observe this in the embryos injected with caffeine. The 1mM concentration caused deformities that led to the eggs clumping and embryo formation did not begin, as in Figure (3-B). It was noted that in the deformed eggs injected with a 5mM concentration, an increased concentration of caffeine led to the eggs appearing in the shape of a pink ball (3-C).



3 (a) 3(b) 3 (c)

Figure 3 Embryos after 14 days (A - Embryos not injected with caffeine, B - Embryos injected with 1Mm of caffeine C - Embryos injected with 5Mm of caffeine).

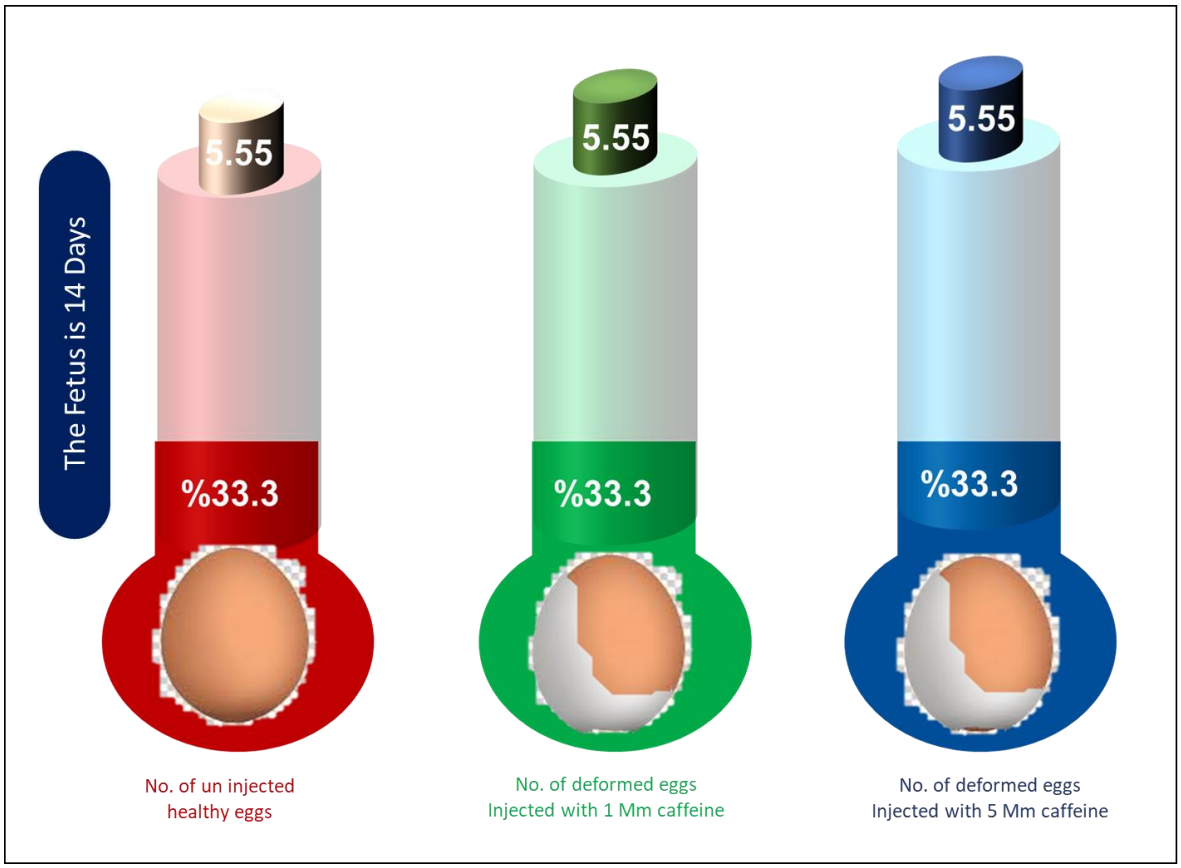
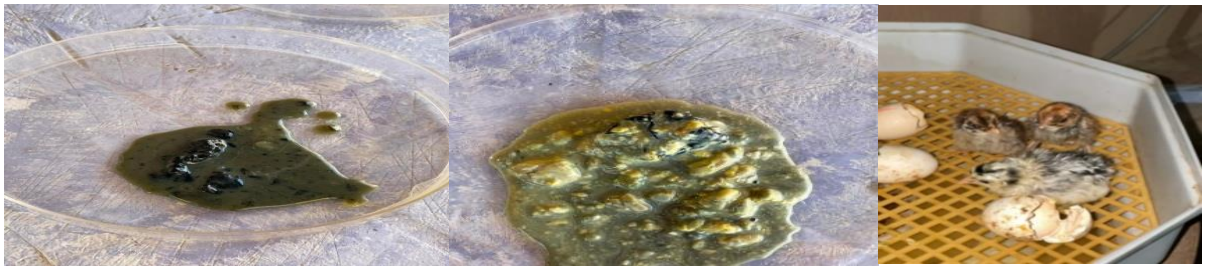


Figure 4 The difference in the rate of deformities between the group of native eggs not injected with caffeine, the deformed eggs injected with 1 mM caffeine, and the deformed eggs injected with 5 mM caffeine at the age of 14 days.

In the control samples, we notice the appearance of limbs and spines and the presence of clear wings, legs and claws with no trace of deformities. Compared to the deformed eggs injected with 1 mM,

deformities occurred that led to the eggs clumping, and the deformed eggs injected with 5 mM increased concentration of caffeine led to the eggs appearing in the form The ball is pink.

Embryos after 21 days: It was observed that in the embryos not injected with caffeine (control samples), the embryos became fully formed and the stage of shell pecking was completed and they hatched and the chicks emerged intact as in Figure (5-A). We note that the embryos injected with caffeine at a concentration of 1Mm had significant deformities that led to their dissolution. Eggs, the absence of blood vessels, and the absence of embryonic formation, as in Figure (5-B), and the embryos injected with caffeine at a concentration of 5 mm The concentration was very strong, leading to the death of the embryos and their appearance in black due to the absence of blood vessels and the lack of blood flow in them, as in Figure (5-C).



5 (a) 5(b) 5 (c)

Figure 5 Embryos after 21 days (A - Embryos not injected with caffeine, B - Embryos injected with 1Mm of caffeine, C - Embryos injected with 5Mm of caffeine).

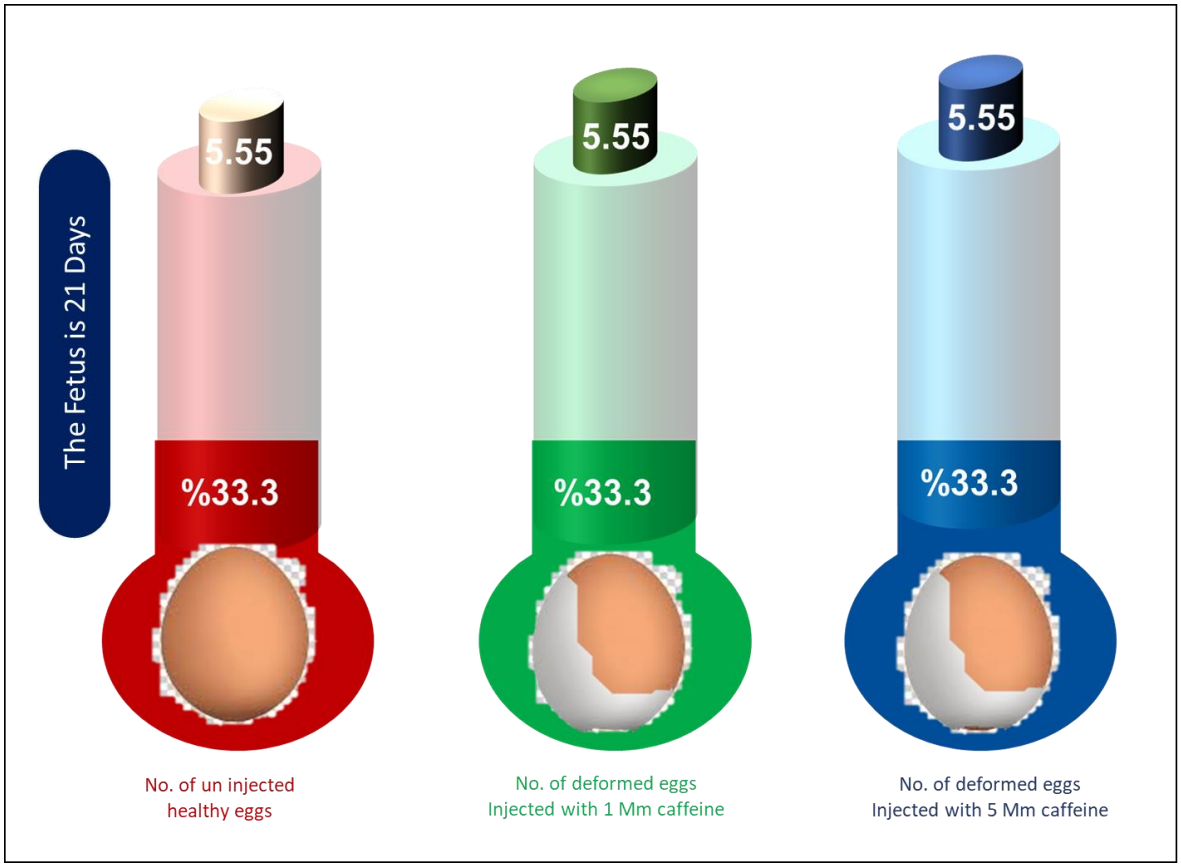


Figure 6 The difference in the rate of deformities between the group of native eggs not injected with caffeine, the deformed eggs injected with 1 mM caffeine, and the deformed eggs injected with 5 mM caffeine at the age of 21 days.

We notice that in the control samples, the eggs hatched and came to life and appeared healthy, compared to the deformed eggs injected with 1 mM, causing major deformities that led to the dissolution of the eggs, and those injected with 5 mM led to the death of the embryos and their appearance in black.

The results of this study agreed with [13] that the higher the caffeine concentration, the greater the fetal deformities. They also agreed with [16] that the excess concentration of caffeine causes delayed growth and death of the fetuses, as it was found that there was an increase in the death rate and the fetuses that survived A woman alive had delayed growth, as agreed with a study by [4] on the effect of caffeine on fetal death, as increasing caffeine consumption during pregnancy leads to an increased risk with increased cups of coffee per day, as it was found that there is a high risk of fetal death.

This study did not agree with the study [12] where the injection was done after 7 days of incubation, as its results showed changes in the thickness of the cornea.

Conclusion:

We conclude from this study the following:

1- Caffeine has a strong effect on the beginning of the embryonic development of local chickens, represented by delayed fetal growth and leading to major deformities that cause the limbs and blood vessels to not appear.

2- The effect of caffeine is more dangerous in the early stages of embryogenesis, so caffeine concentrations must be reduced during the early stages of embryogenesis.

3- There is a direct relationship between caffeine concentrations, fetal malformations, and developmental delays. The higher the caffeine concentration, the greater the fetal malformations and the cessation of fetal formation.

4- Caffeine leads to the delay and cessation of embryonic development in local chickens and the occurrence of fatal deformities that lead to the death of embryos inside the eggs and the failure to form normal embryos.

Recommendations:

Through this study, we recommend conducting such studies at different ages, under different conditions, with different concentrations of caffeine, and reaching a wide range to reduce genetic abnormalities and promote healthy fetal growth.

We also recommend reducing the use of products containing concentrations of caffeine due to its effect on the pregnant woman and the proper development of the fetus.

We also recommend providing integrated laboratories and providing tools and equipment for students to conduct broader studies within the scope of this subject.

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