Альсаму Уля
Студент магистратуры, Факультет наук
Кафедра биологии, Университет Аль-Баас
Хомс, Сирия
Альали Мухиддин
Профессор факультета наук
Кафедра биологии, Университет Аль-Баас
Хомс, Сирия
Хамуд Самир
Профессор факультета наук
Кафедра биологии, Университет Аль-Баас
Хомс, Сирия
AL Samoo Ola
Master student, Faculty of Sciences
Department of Biology, Al-Baath University
Homs, Syria
AL Ali Mohedeen
Prof. Faculty of Sciences,
Department of Biology, Al-Baath University
Homs, Syria
Hamoud Samir
Prof. Faculty of Sciences
Department of Biology, Al-Baath University, Homs, Syria

EFFECT OF ETHANOL CONCENTRATIONS ON THE THREE DERMIS CHICKEN EMBRYOS DEVELOPMENT DURING PERIOD EARLY ORGANOGENESIS

Abstract: *The study was conducted on 480 sterile fertilized eggs (Rus 306 strain), distributed in 4 groups at a rate of 120 eggs in each of them, incubating the*

eggs until the Definitive primitive streak stage period (18 h). the experimental groups (1,2,3) were injected with the ethyl alcohol solution under the Blastodisc in the dose was 0.3 ml and in concentrations (7% -10% -14%). leaving half of the fourth group eggs (control) without opening and injecting the other half with the carrier (physiological solution).

Egg openings were closed and re-incubated; embryos were extracted in evolutionary stages 28,38 hours incubation.

Alcohol showed various effects on the development of chicken embryos, ranging from the stimulating effect with a low concentration (7%), to the appearance of some developmental deformation and anomalies under the influence of the medium concentration (10%), and the death of embryos at higher concentrations.

The results indicated that alcohol has an effect: teratogenic with moderate concentrations, fatal with high concentrations and activated with low concentrations.

Key words: *Blastodisc, ethyl alcohol, Definitive Primitive Streak.*

Влияние концентраций этанола на развитие трех дермы у куриных эмбрионов в период раннего органогенеза.

Аннотация: *Исследование проводилось на 480 стерильных оплодотворенных яйцеклетках (штамм Rus 306), распределенных по 4 группам из расчета по 120 яиц в каждой из них, инкубируя яйца до конечной первичной линии в течение периода (18 ч), опытным группам (1,2,3) вводили раствор этилового спирта под Оригинатором диска в дозе составлял 0,3 мл и в концентрациях (7-10% -14%), оставляя половину яиц четвертой группы (контроль) без вскрытия и вводя в другую половину носитель (физиологический раствор).*

Отверстия для яиц закрывали и повторно инкубировали, эмбрионы извлекали на эволюционных стадиях инкубации 38,28 часа.

Алкоголь проявлял различные эффекты на развитие куриных эмбрионов, начиная от стимулирующего эффекта с низкой концентрацией (7%), появления некоторых отклонений в развитии и аномалий под влиянием концентрации среды (10%) и гибели эмбрионов при более высоких концентрациях.

Результаты показали, что алкоголь обладает эффектом: тератогенный при умеренных концентрациях, смертельный при высоких концентрациях и активированный при низких концентрациях.

Ключевые слова: *Оригинатор диска, этиловый спирт, конечная первичная линия.*

1-Introduction:

It solved many of the problems that the pregnant mother was exposed to with the progress of science and development, but new problems emerged, the most important of which is the vast amount of medicinal and chemical medicinal materials that are used today to treat diseases as they enter into the composition of many drugs and preparations used.

Most medications and chemical compounds to which a pregnant woman is exposed can pass into the fetus unless they are vandalized or altered during the transit. The distorting effects of many drugs and chemicals are related to the dose and duration of exposure to teratogenic substances during pregnancy, for example, the human fetus is most likely to be deformed before the eighth week of pregnancy.

Alcohol is one of the chemical factors that can easily pass the placenta barrier and reach the fetus, causing abnormalities, often represented by Fetal alcohol syndrome (FAS), which pretends to be delayed before and after birth, represented by cranial anomalies, central nervous system weakness, and defects including Musculoskeletal system, heart, eyes, and kidneys [3,2,18]. The toxic effects of ethanol on the development of embryos are more evident when consuming ethanol during the stage of organogenesis [1,24]. As this period corresponds to a person in the third week until eighth of pregnancy and is called the critical period that period is short and does not last long in chicken embryos, ranging from 18-36 hours incubation [1,25].

Some studies indicated that exposure to increasing doses and concentrations of ethanol has morphologically distorting effects, and showed that the severity of the

damage depends on the amount and timing of exposure to it[1].

Knowledge of embryo and neonatal changes affected by fetal alcohol syndrome was the aim of the study [19], which was characterized by delayed growth and characteristic facial abnormalities. The same study confirmed that ethanol at a very high dose leads to 6-10% of fetuses developing fetal alcohol syndrome.

Studies on the relationship between the effect of ethanol and the pulse rate in the advanced stages of fetal development have shown that alcohol in low concentrations leads to a high pulse rate in the first days and then the death of fetuses, while the high concentrations were closely related to the control group [2]. While another study conducted by him [3] on the heart rate of the fetus treated with alcohol showed that, the heart rate increased at a random frequency with high concentrations.

The same aim (the effect of ethanol on the heart rate) was studied, but using another animal model in an experiment he conducted [22] on Daphnia (zooplankton spreading widely in channels and waterways) which showed that increased ethanol concentration led to a lower heart rate.

The study, [5], aimed to know the effect of ethanol on vascularization, as high concentrations were severely inhibited and vascular morphology, but no effect of low concentrations was observed.

Another study [4] indicated the effects of alcohol exposure, which were emansio growth, structural abnormalities, heart abnormalities, and delay in neural tube closure.

The effect of different concentrations of ethanol on the survival rate of fetuses [5] conducted a study where the death rate of fetuses treated with high concentrations was 100%, while the percentage of deaths was limited to 64% during treatment with medium concentrations.

]20 [touched on the same aim (survival rate) in his study, which confirmed that the high concentrations of alcohol (20%) led to a decrease in the survival rate of fetuses compared to control groups.

It was previously indicated [6] that high concentrations of ethanol programmed cell death (apoptosis) and retardation cellular migration. The results of the study conducted by him [21] also showed that exposure to ethanol before birth leads to apoptosis including neural crest cells (which are of great importance as they are precursors to a wide range of cells and neurological structures).

]7 [In his study of the stages of embryo development، he emphasized that ethanol inhibits the natural development of the central nervous system، and the essence of this effect lies in impeding the proliferation of glia cells (supportive and nourishing to nerve cells)، and that the time of exposure to alcohol determines the extent of the risk resulting from it.

One of the most important developmental stages is the early stages of development of a chicken embryo, in which all the processes that will later Formation all the tissues and organs of the body (cellular migration –

] . 1 [organogenesis) The 8 [It is stated that the focus of attention in studies revolves around the effect of high concentrations (14% and 16%) of ethanol، comparator to low concentrations. In another study in the same way, it showed the effect of high concentrations that led to reduced fetal weights [9]. He also noted [10] the importance of using a chicken embryo as a model for studying fetal alcohol syndrome. Which studies have shown that the effect of fetal alcohol syndrome on chicken embryos is similar to those in human embryos. [1]

A study conducted by [13] concluded that fetal abnormalities that occur in the advanced stages of embryo development may be due to exposure to the teratogenic effect of alcohol during the development of the neural tube. Another study focused on the effect from the cellular side, and found that exposure to alcohol leads to reduced proliferation cell, inactivation of DNA and protein synthesis, and programmed cell death [14].

The oxidative stress caused by the effect of alcohol during fetal development was the focus of the study [15], which concluded that oxidative stress disrupts mitochondrial functions, resulting in nervous disturbances and causative elevation fetal abnormalities and a high level of apoptosis.

The results of research conducted by [23] on the mouse embryos through the dose of the pregnant mother, showed that ethanol leads to disorders in fetal development due to oxidative stress.

He continued [16] in the same path to study the effect of oxidative stress caused by alcohol, and showed that when oxidative stress is high in tissues, cells can't detoxification, which leads to the destruction of Cytoskeleton and reduces cell adhesion, and hence cell death.

Given that the available research did not touch at all to study the early stages of fetal development

2-Research Aims:

1-To know the effect of different concentrations of ethyl alcohol on the early stages of chicken embryo development.

2-Observing possible developmental defects and anomalies in chicken embryos under the influence of ethyl alcohol injected directly beneath the embryonic disk.

3-materials and methods

The experiment was conducted on 480 fertilized eggs (Ross 306 strain). They were distributed in 4 groups at a rate of 120 eggs in each, incubating the eggs under a temperature of 37.5-38 ° C and a relative humidity of about 60%, leaving half of the eggs of the fourth group (the control) without opening and the other half was injected with the physiological solution as the carrier of the active substance.

Experiment groups (1,2,3) were injected with alcohol under the embryonic disc at the definitive primitive streak stage 18 h incubation taking into account the necessary sterilization conditions (Figure1).

Figure 1: photo microscopic view of a whole surface view of a 18 hour chick embryo incubation,40X magnification of hematoxylin-eosin tincure.

Notes at this stage form Alajasi disk embryogenesis, the arrival of the primary line to about two-thirds of the region and constitute the beginning of the layer vertical elongation.

Group I eggs (1) were injected with 0.3 ml of 7% alcohol. While eggs of the second group (2) were injected with 0.3 ml of 10% alcohol, and eggs of the third group (3) with 0.3 ml of 14% alcohol.

After the injection process was completed, the cut portion of the lime scale was returned, the egg openings closed with sterile medical plaster, the eggs were rotated 180 degrees and returned to the nursery to continue the lap.

Then the eggs were opened and the embryos were extracted for examination in the stages of 28,38 hours incubation and with an effect time of (10-20) hours in sequence. Embryonic samples were treated using conventional methods, and stained with hematoxylin-eosin (H&E).Embryo preparations were studied microscopically, results and abnormalities were recorded according to the required stages.

4-Result and Discussion: The injection of chicken embryos at a low concentration of ethanol 7% stimulated the development of embryos, as the embryos were previous in their development to the age of the control (28 hours incubation). A greater number of somites were recorded, and a clear development of the brain region and in the formation of both cardiovascular anlage, compared to the control.

The normal development of control embryos is injected only with the physiological solution and the appearance of four pair of somites with the beginning of angiogenesis. (Figure2)

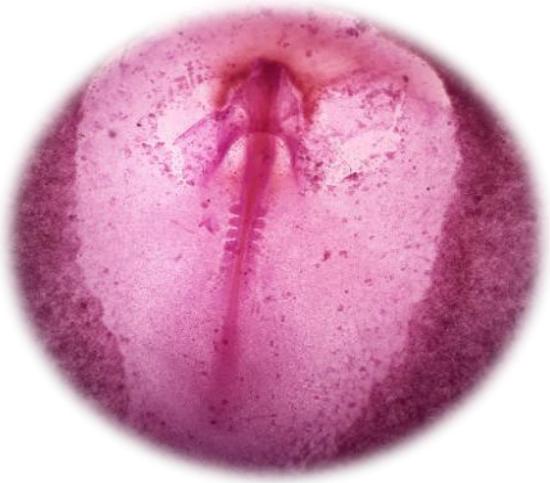

Figure 2: photo microscopic of a wole surface view of a 28-hour embryo incubation injected at 18 o'clock with a physiological solution, 40x magnification of H&E dye.

It is observed through the results that the 7% alcohol-treatment embryos (Figure 3) are more developed than the control embryos (Figure 2) in terms of the number of somites, the evolution of the Brain vesicules, and the development of Harte anloge.
By calculating the number of somites in experimental embryos, we find 12 pair of somites, and depending on the number in this case, the age of the embryos is approximately 33 hours incubation, i.e. 5-6 hours greater than the control stage (four pair of the somites). (Figure3)

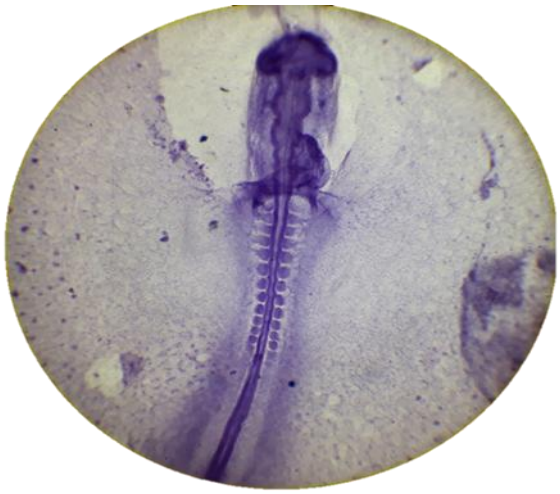

Figure 3: Microscopy of a superficial view of a 28-hour injected fetus at 18 o'clock with a concentration alcohol of 7%, 10 x 10x Dye H&E

The results showed that the injection of alcohol with intermediate concentrations led to the emergence of disturbances in the development of embryos at the age of 28 hours incubation, in the form of delay in growth, anomalies in the forms of somites or their loss. lack of growth and development of the brain vesicules and, cardiovascular anloge compared with the embryos of groups with low concentrations and control of embryos. Figure 4

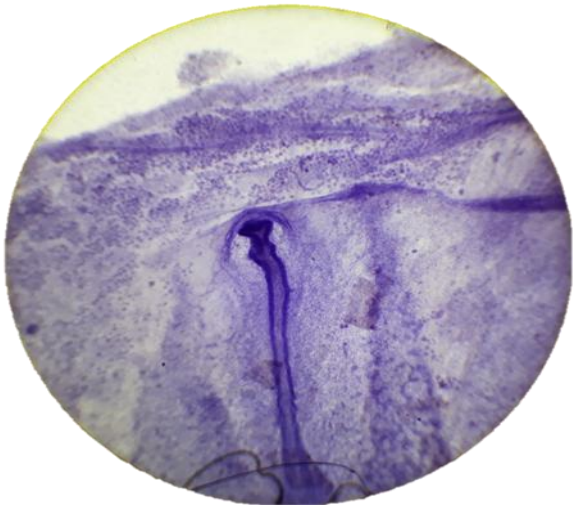

Figure 4:photo microscopic view of a surface view of a 28-hour-old chicken embryo with a brood injected at 18 o'clock with an alcohol concentration of 10%. Zoom 40X H&E dye.

When comparator Figure 4 with the control group (Fig. 2) and embryos injected with low concentrations (Figure 3), the cacogenic effect of alcohol at medius concentrations of 10% is evident, as deformation of the development of the brain vesicules with the presence of Zakzak in the neural tube region in addition to abnormalities in the forms of somites.

While the results of high-dose 14% injection showed in the 28-hour incubation stage, embryos shapeless, as there was no differentiation of the brain region and the region of the stem (Figure 5), while a high percentage of Blastodisc were without embryos. (Figure6)

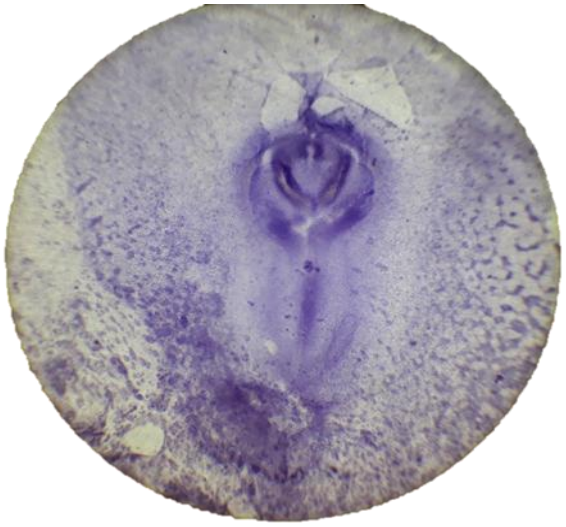

Figure 5: photo microscopic view of a whole surface view of a 28-hour-old chicken embryo. Brood injected at 18 o'clock with an alcohol concentration of 14%. 40x Dye H&E.

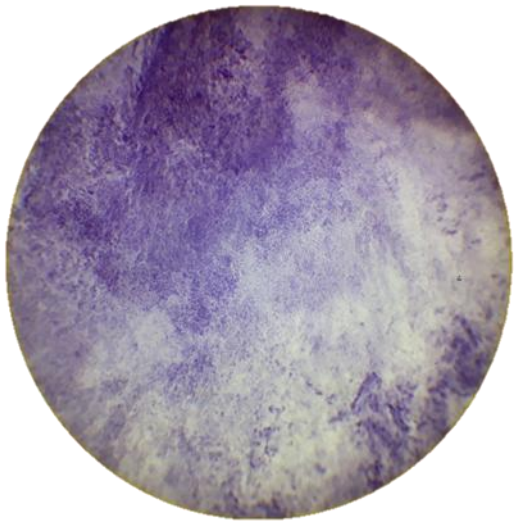

Figure 6: photo microscopic of a embryonic disc without a embryo injected with 14% alcohol. 40 x H&E dye.

Embryos of 38 hours showed a incubation injection with a low concentration of 7%, as it showed a slight activation in the development of the embryo compared to the control (Figure 8.7), where a simple development in the Brain vesicules is observed compared to the control, in addition to a number of more than 3- 4 somites.

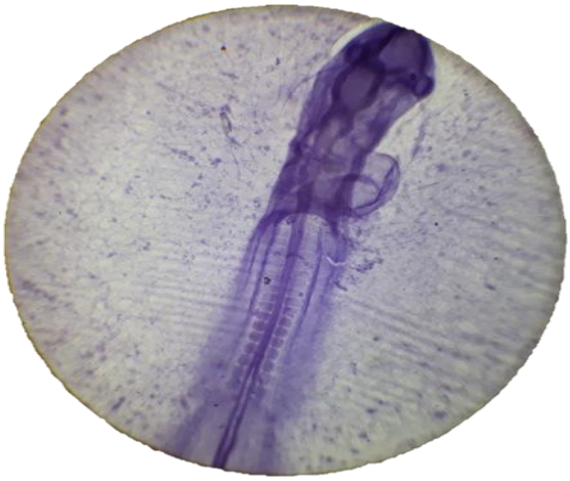

Figure 7:photo microscopic of a whole surface view of a 38-hour embryo incubation injected with the physiological solution. 40 x H&E dye

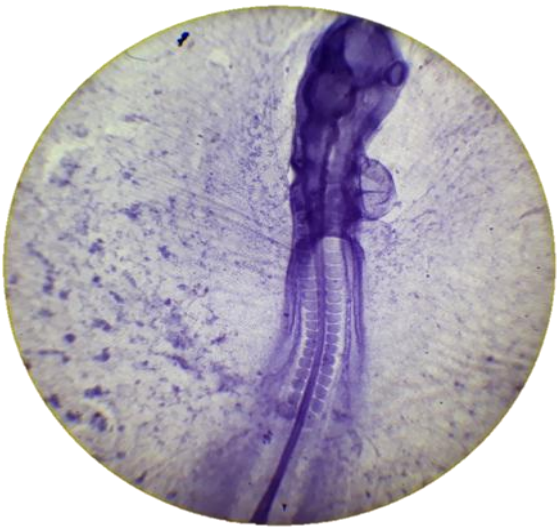

Figure 8: photo microscopic of a surface view of a 38-hour-old embryo injected at 18 o'clock with a 7% alcohol concentration. 40 X, H&E dye.

Anomalies were observed with the forms of somites, atrophy of the Brain region and lack of development of cardiac anlage in embryos of 38 hours, incubation injection, at an medius concentration of 10%, compared with the control. Figure 9

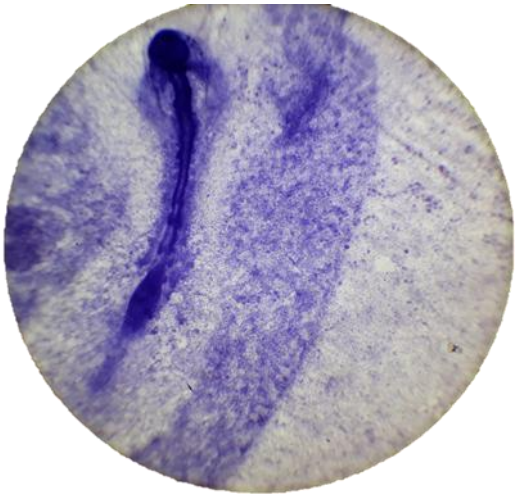

Figure 9: photo microscopic of a whole surface of a 38-hour-old embryo incubation injection at 18 o'clock with alcohol Concentration of 10% . 40 X, H&E.

While no embryos were found within, the embryonic discs injected with high concentrations of 14% alcohol (Figure 10).

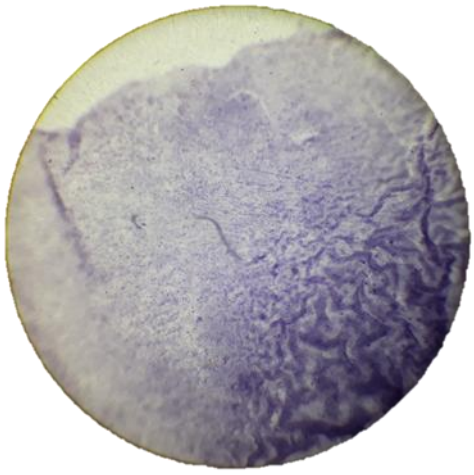

Figure 10:photo microscopic of embryonic disc without embryo injected with a 14% alcohol. 40 X, H&E dye.

A lot of researches have examined the effects of alcohol exposure that is fetal alcohol syndrome that has been described in more than one study [12,11]. However, no research has been mentioned or conducted on early developmental stages, especially in the stage of organogenesis. Some studies indicated the presence of defects in the nervous system due to the influence of alcohol (14%, 16%) injected into the air chamber [8,7]. This is consistent with the current study that demonstrated the presence of abnormalities and lack of development of the Brain vesicules when treating embryos with medius concentrations (10%). Upon returning to the derivatives of the embryonic dermis, it becomes clear that the paraxial mesoderm is the axial neighbor that gives the

somites (Dorsal segmental mesoderm). It is well known that each somite is divided into the sclerotomy and derma myotome, which form the vertebral column and the muscular vertebrae in the trunk region. Where the results of this study confirmed the presence of abnormalities in the forms of somites and their numbers or complete absence when treating embryos with 10% alcohol. This corresponds to some studies that indicated structural deformities[12,11,4].

The blood vessels and their formation were of interest in many studies [5] that demonstrated that blood vessels did not formation due to the effect of high concentrations (10%, 13%, 15%), and this is consistent with the results of this study, which showed that blood vessels did not form under the influence of medium concentrations 10%. When comparing the results of the study [5] and the current study it is found that the effect of high concentrations have equivalent to the effect of medium concentrations and this may be due to the difference in the location of the injection as most studies among them [5], were injections in the air chamber while the injection was made in the current study under the embryonic disc Directly, which leads to a greater effect of alcohol, it may be equivalent to the effect of high concentrations injected into the air chamber.

]7,1 [indicated that the effect time and timing of exposure to alcohol determines the severity of alcohol and this may explain the difference between previous studies and the current study as the injection was carried out at an 18-hour incubation stage and the effect time was 10,20 hours compared to a study [5,4,3,1] which It was treated before cuddling and with an impact time of several days.

]2 [In his study of the effect of different concentrations of alcohol، he indicated that low concentrations led to a high pulse rate in fetuses (embryo stimulation) and this is consistent with the results of this research، which confirmed that in low concentrations the fetus is stimulated and pre-age compared to the control.

The results of the current study, which showed the stimulating effects of low alcohol concentrations, did not coincide with many previous studies that concluded that low alcohol concentrations are not effective in development [2,7,8], which may explain the different injection conditions, its location and age. As a result, not all

previous research deals with the study of the early stages of development of the chicken embryo, as well as the location and timing of the injection that was carried out in these experiments, under the embryonic disc and the stage of the 18-hour incubation (Definite primitive streak).

5-Conclusions: 1- Teratogenic effects appeared when using medius concentrations of alcohol with abnormalities in the shape or absence of somites, disturbances and delays in the development of the Brain vesicules and vascular formation.

2-The stimulating effect of alcohol in low concentrations with increasing number of somites represented a clear development of the Brain vesicules, vascularization and cardiac anlage compared to the control of the same age group.

References:

1-Janowicz, M (2013) - effect of ethanol dosage and ascorbic acid antioxidant therapy on chick development .Bio 489.

2-Blumenthal, A (2015) - ethanol exposure on pulse in chick embryo Bio254. - 3-Mctaughlin, J (2006) - determining the effects of ethanol on the 5-day chicken embryo heart rate.Bio240W.

4-Gouukhast, B (2017) - effects of alcohol during secondary deurulation in chick embryos .Dirges 7:227-235.

5-Laghri, Z; Samo, A; Waryani, B (2015) - effect of a single dose of ethanol on survival rate and angiogenesis of chick embryo.vol.3 no1-pp. 8-11.

6-Smith, S; Garic, A (2014) - neural crest development in fetal alcohol syndrome.102 (3):210-220.

7-Bupp, B; Shibly, IA (1998) - terogenieity of ethanol in different chicken strains.Alcohol & Alcoholism vol33.No5, pp. 457-464.

8-Costal, G; Shipley, IA (2002) - inhibition of muscarinic receptor induced proliferation of. Astroglial cells by Ethanol: Mechanisms and Implications for Fetal Alcohol Syndrome. Neurotoxicology (23): pp. 685-691.

9-hartlm, W; Shibly, IA (2002) - supraphysiological acetaldehyde levels suppress growth of chicken embryos. Alcohol (28):pp111-115.
10-Kiecker, C (2016)-the chick embryo as a model for the effects of prental exposure to alcohol on craniofacial development.Bio415 pp314-325.
11-Jennifer, D; Kenneth, R (2010) - fetal alcohol spectrum disorders.vol.33 pp118-126.
12-Shannon, E; Timothy, A (2011) - the use of animals for the study of fetal alcohol spectrum disorders. Alcohol research &Health vol34.No1, pp. 92-98.
13-Joneskl; Smithdw (1973) - Recognition of fetal alcohol syndrome in early infancy. Lancet; 302 (7836):999-1001.
14-Anthonyb (2008) - Alcohol exposure alters cell cycle and apoptotic events during early neurulation. Alcohol;43(3):261-73.
15-Zhaoz; Eckertrl; Reeceea.(2012) - Reductionin embryonic malformation and alleviation of endoplasmic reticulum stress by nitric oxide synthase inhibition in diabetic embryopathy. repord Sci 2012; 19(8):823-831.
16-Zhoul; Yueby (1999) - Oxidative stress affects cytoskeletal structure and cell-matrix in cells from an ocular tissue: the trabecular meshwork. J cell physical 1999; 180(2):182-189.
17-Jarvityd (2006) - the effect of ethanol concentration on developing chicken embryos. Marietta college .pp. 1-13.
18-Welch-Carre E. 2005.The Neurodevelopment consequences in prenatal alcohol exposure. Advances in Neonatal care (5): pp. 217-229.
19-ORNOY, A; ERGAZ, Z (2010)-Alcohol Abuse in pregnant women: effects of the fetus and newborn, mode of action and maternal treatment. Journal of environmental research and public health, 7,364-379.
20-KAMRAN, K; KHAN, L (2009)-effect of ethanol vapour exposure on survival of chick embryos. Journal of the college of physicians surgeons 19(3):150-153.

21-GARIC, A; AMBERGER, ED (2011)-CaMKll activation is a noval effector of alcohol's neurotoxicity in neural crest stem/progenitor cell. Journal neurochemistry 118:646-657.
22-PARADEL, I; VICTORIA, A; CANALITA, P (2015)-study on the effect of alcoholic beverages on the heart rate of daphnia (daphnia magna).biological and chemical research 3,215-221.
23-WENTZEL, P; ERIKSSON, U (2006)-Ethanol-induced fetal dysmorphogenesis in the mouse is diminished by high ant oxidative capacity of the mother.92 (2), 416-422.
24-Parnell SE, Dehart DB, Wills TA, ChenS, HoCW.2006.Maternal oral intake mouse model for fetal alcohol spectrum disorders: ocular defects as a measure of effects.Alcoholism: Clinical and experimental research 30(10):1791-1798.
25-Smith S.1997.Alcohol-induced cell death in the embryos.Alcohol Health and Research World 21(4):287-297.