The Effect of Alcohol on the Early Stages of Chicken Embryonic Development

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SUMMARY

The aim of the investigation was to find out whether alcohol had any effects on chicken embryos during their early stages of development. The nature and extent of these effects were investigated to identify the concentration above which causes death of the embryo.

Hypothesis: The presence of ethanol (alcohol) will have an effect on the embryo's development during the first two days. Biological damages and reduction of body length is expected to be the result of ethanol exposure to the embryo.

The results showed that alcohol had an effect on the development of chicken embryos in the following ways: reduction of embryonic body length, higher mortality rate and inhibition of heart development. Also, as the concentration of ethanol exposed to the embryos increased, the severity of the effects increased too. An estimated concentration of 1% ethanol was concluded as a lethal concentration that causes the complete death of the chick embryo.

INTRODUCTION

The aim of the investigation was to find out whether alcohol had any effects on chicken embryos during their early stages of development. The nature and extent of these effects were investigated to identify the concentration, above which causes death of the embryo.

Hypothesis: The presence of ethanol (alcohol) will have an effect on the embryo's development during the first two days. Biological damages and reduction of body length is expected to be the result of ethanol exposure to the embryo.

These assumptions were based on the studies of Fetal Alcohol Syndrome (FAS) that exist amongst the children of alcohol-dependent women.

Alcohol consumption during pregnancy results in spontaneous abortion, growth retardation, facial abnormalities, and mental retardation. This collection of defects is known as ***fetal alcohol syndrome (FAS)****. In milder forms, the condition is known as* ***fetal alcohol effects****.* (Cummings 1997)

Alcohol produced its effects by constricting the blood vessels of the placenta and umbilical cord, reducing the supply of oxygen to the foetus. Measurements on placentas recovered from normal births show that umbilical blood vessels constrict in the presence of alcohol concentrations as low as 0.05%, the amount contained in 1 to 1.5 drinks. Oxygen deprivation may play a role in the behavioural defects and mental retardation associated with FAS. (Cummings 1997)

There is great variation in the ability of mothers and foetuses to metabolise ethanol, and it is thought that 30-40% of the children born to alcoholic mothers who drink during pregnancy will have FAS. (Gilbert 2003)

The biological significance of this investigation was to verify the effects of alcohol on chick embryonic development and relate it to alcohol consumption during pregnancy and its associated FAS symptoms.

To investigate the effects of alcohol on foetuses, chicken embryos were used as a substitution for human embryos due to their close biological resemblance. The chicken embryonic development lasts 21 days in total. But this investigation only monitors their growth during the first 2 days due to health and safety regulations.

The Chicken Egg

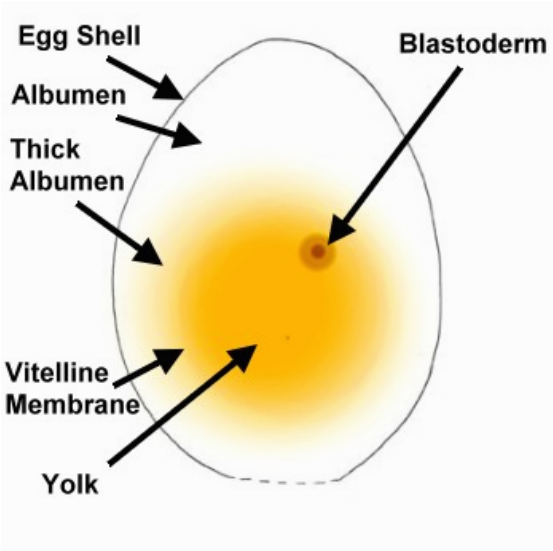


Figure 1

During the first 24 hours of incubation of the chick embryo, the vertebral column starts to develop, followed by the beginning of the nervous system, then the head and the eyes. During the next 24 hours, the formation of the heart and ears begin. The heart starts to beat by the end of the second day. (Smith 1914)

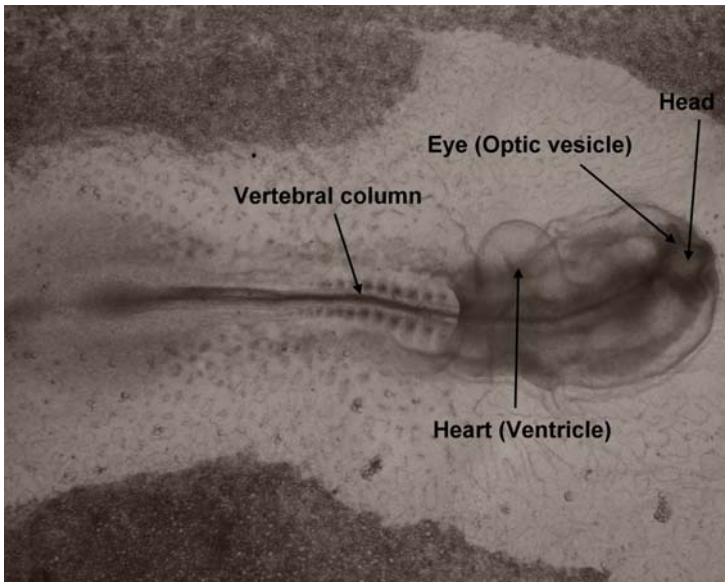


Figure 2
Chicken Embryo at Stage 13 (approx. 42 hours after incubation)

In the presence of ethanol, according to FAS, the embryo should show some abnormalities during the first 2 days. Effects of FAS such as persistent postnatal growth lag maybe recognizable by a reduction in the body length of the embryos.

PROCEDURES

EC (Early Chick) Culture was the technique used to grow the chick embryos. The method provided results that could be photographed and analysed. Before proceeding with this technique. The following preparations were required.

Preparations

Preparation of Saline Solution

See Appendix 1. (p23)

Preparation of filter paper

Apparatus: Whatman filter paper no.5
Hole puncher
Scissors

The Whatman filter paper was cut into numerous 1.5cm x 2.0cm rectangular squares. The hole puncher was used to create a four-leaf clover shaped hole in the centre of each square.

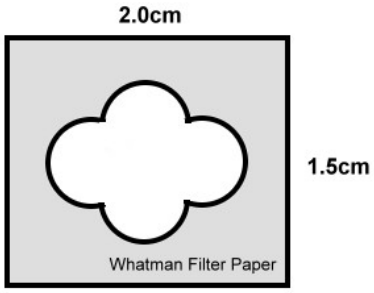


Figure 3
Four-leaf clover shaped Filter paper

Preparation of Agar-albumen culture dishes

See Appendix 2. (p23)

Since the culture dishes can only be stored for up to 2 weeks, a smaller number was prepared each time than the suggested number because only a small number of dishes were required for each experiment. And the time gap between experiments often exceeded 2 weeks.

Approximately 40 culture dishes were prepared each time:

- 68ml of thin albumen, collected from a dozen non-incubated eggs
- 68ml of simple saline
- 0.408g of Bacto-Agar
- 68μl of Penicillin

EC Culture

EC (Early Chick) Culture (with alcohol free culture dishes)

See Appendix 3. (p24)

Adding ethanol

Preparation of Agar-Albumen culture dishes containing ethanol

This was prepared using the same procedures as making a standard Agar-Albumen culture dish, but with the addition of ethanol. To make 34ml of 0.1% ethanol Agar-Albumen solution and 34ml of 1% ethanol Agar-Albumen solution:

68ml of thin albumen was collected from a dozen eggs and kept in a water bath at 49°C.

0.408g of Bacto-Agar was added to 68ml of simple saline and mixed together in a beaker. A magnetic stirrer with an integrated hot plate was used to mix the solution to ensure that the Bacto-Agar dissolves. The hot plate was turned on to assist this. (Bacto-Agar is a very insoluble solidifying agent in which extraveous matter, pigmented portions and salts have been reduced to a minimum.)

When the solution turned clear from the originally misty beige colour, it showed that the Bacto-Agar has dissolved completely in the saline solution. This required approximately 5 minutes.

The volume of solution was re-measured because some of the solution had evaporated due to heat of the hot plate. The new volume in this case was 60ml. 8ml of saline solution was added to make up to 68ml.

- 68µl of ethanol was added to 34ml of the solutions.
- 680µl of ethanol was added to 34ml of the solutions.

The opening of the measuring cylinder was ceiled and both solutions were inverted 5 times. 68µl and 680µl of ethanol will eventually produce a 0.1% and 1% ethanol Agar-Albumen solution respectively. The measuring cylinders were labelled with the percentages to avoid confusion.

The thin albumen was taken out of the water bath and added to dissolved Bacto-Agar solutions i.e. 34ml to each concentration of solution. 34µl of Penicillin was also added to each solution using a 100µl pipette. Swirling gently on a magnetic stirrer for 60 seconds mixed the solutions. Then they were left in the water bath to prevent solidification whilst 40 35mm sterile petri dishes were laid out on a flat surface. 20 petri dishes were used for each concentration.

1.5ml of the agar was pipetted into each petri dish without introducing air bubbles as this may interfere with the development of the embryos. The air bubbles were avoided by pipetting out the agar slowly at the start with the endpoint of the pipette just touching the bottom surface of the petri dish.

Each petri dish was swirled around once to create a thin layer of agar, spread out evenly, covering the bottom surface of the petri dish. The petri dish lids were replaced and the agar was stored at 4 under sterile conditions for usage within 2 weeks.

Experiments

For each experiment, a number of eggs were processed using the technique EC culture. But not all the embryos reached the stage of incubation due to varies reasons:

- Irregular looking blastoderms were disregarded because they were likely to undergo abnormal embryonic development.
- Vitelline membrane ripped very easily, often before transferring the blastoderm onto the filter paper. As a result, the blastoderm was swept away by the flow of yolk and lost.
- Whilst washing off the yolk in saline solution, the blastoderm was often damaged.
- Experimental errors i.e. inaccurate measurements.

		Number of eggs processed	Number of eggs successfully grown/processed *
Experiment 1	Control	24	17
	0.1% ethanol	6	3
	1% ethanol	12	7
Experiment 2	Control	6	6
	0.1% ethanol	6	4
	1% ethanol	6	3
Experiment 3	Control	6	4
	0.1% ethanol	6	3
	1% ethanol	24	21

Table 1
Description of number of eggs for each experiment
* Then incubated for 30 hours.

RESULTS

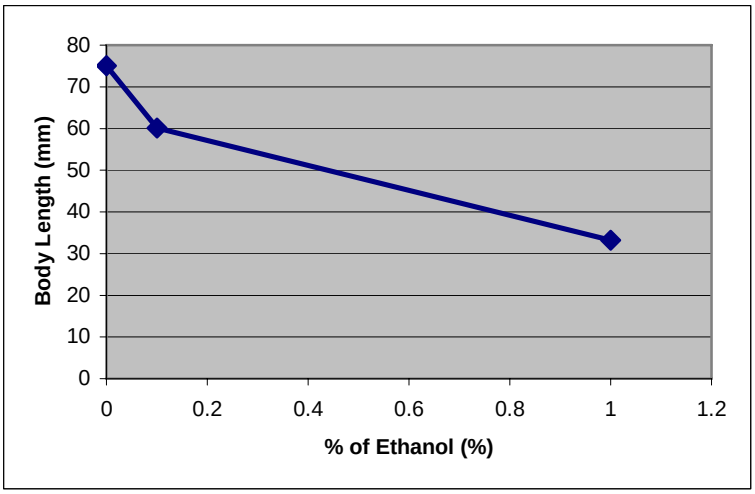
Body Length

The embryos were photographed using a camera that was connected to a microscope and a computer. The microscope magnified all the embryos the same number of times and was then photographed using the camera. The photos obtained were printed using a constant scale and was measured using a regular ruler in millimetres for comparison of body length.

% of Ethanol \ Body length (mm)	Experiment 1	Experiment 2	Experiment 3	Overall Average
0%	74.1	80.7	75.1	75.1
0.1%	53.5	70.3	59.7	60.1
1%	21.7	28.0	36.0	33.2

Table 2
Body length of embryos

The results shown in Table 2 shows that the presence of ethanol in the agar, which supplied the embryos with nutrients, did have an effect on their growth and development. 0.1% ethanol reduced their body length by an average of 20% and 1% ethanol reduced their body length by an average of 56% in comparison to embryos grown under alcohol-free conditions. Hence, as the concentration of ethanol increases, the body length of embryo decreases. (Graph 1 on page11)



Graph 1
The Relationship between % of ethanol and Body Length of Embryo

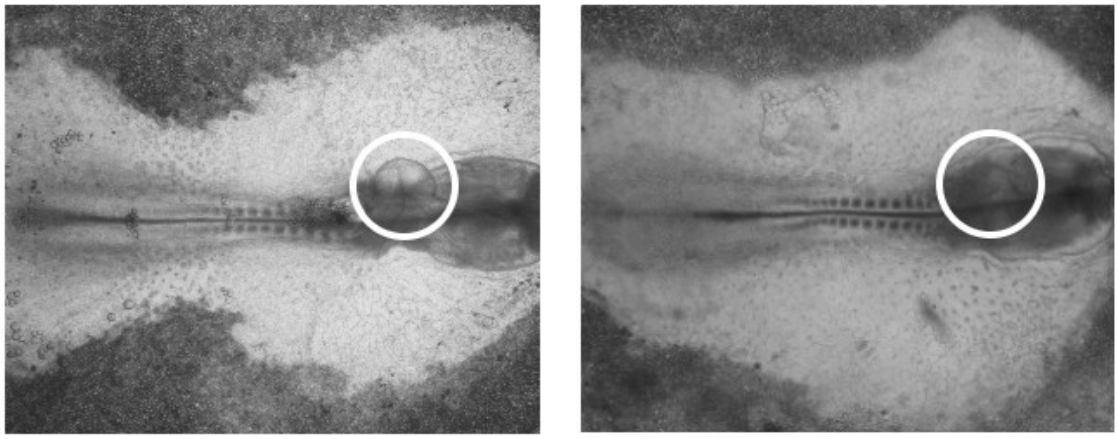
Effects	**Percentage of embryos showing effect (%)**											
	0% ethanol (control)				0.1% ethanol				1% ethanol			
	Experiment number											
	1	2	3	**Average**	1	2	3	**Average**	1	2	3	**Average**
Heart development observed	76	100	29	**67**	17	75	0	**31**	0	0	0	**0**
Slow growth rate	12	0	0	**7**	50	25	100	**54**	100	100	100	**100**
Inhibition of body parts formation	18	0	0	**11**	33	25	0	**23**	50	33	19	**25**
Death	0	0	0	**0**	0	0	0	**0**	25	33	24	**25**
Division of Spinal chord and somites	0	0	0	**0**	17	0	0	**8**	0	0	0	**0**
Twins	0	0	0	**0**	0	25	0	**8**	0	0	0	**0**

Table 3
Effects of ethanol on embryos

Heart Development

The heart of chick embryos developed under alcohol free conditions and chick embryos of which were subjected to ethanol were compared. It can only develop when the embryo reaches a certain stage in its development (stage 12 of "Normal Stages of Chick Embryonic Development" See p.20). Therefore, its presence or absence can be used as an indicator of the embryos' rate of growth. An embryo with a developing heart means it is further developed than an embryo without one. Consequently, the higher the percentage of heart development, the faster the rate of embryonic growth.

Table 3 show that the alcohol free embryos have a higher percentage of heart development than embryos that were in contact with ethanol. This suggests that alcohol has had an effect on the embryos rate of growth.



0% ethanol - heart present

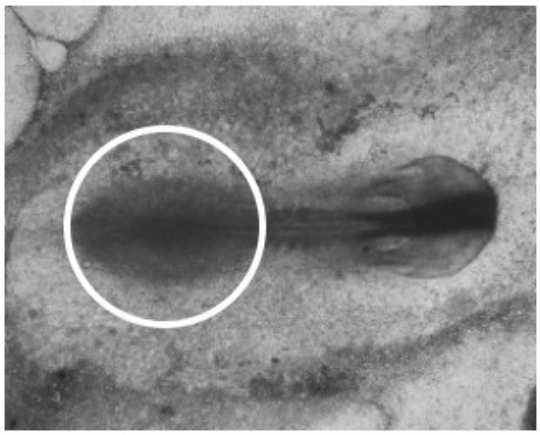
0.1% ethanol - heart absent

Figure 4
Effect of ethanol on heart development

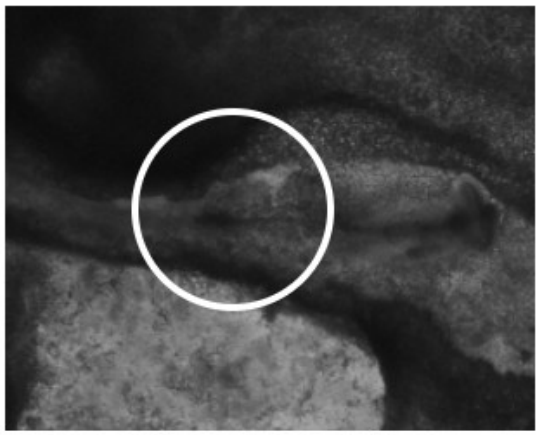
All photographs show embryos at x magnification.

Inhibition of Body Parts Formation

These embryos have missing body parts excluding the heart. The heart was monitored seperately. Such embryos will eventually die (before birth). The results in Table 3 showed an increase in the occurance of missing body parts as the concentration of ethanol subjected to the embryos increased.



0% ethanol



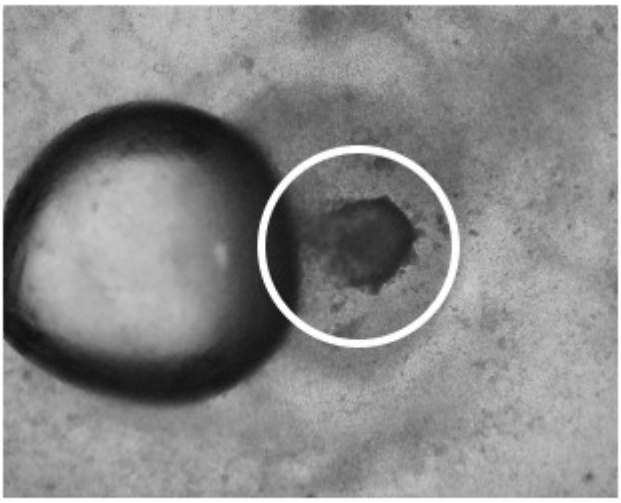
1% ethanol - bottom half of embryo missing

Figure 5
Missing embryonic body parts

Death

The mortality rate for embryos developed under alcohol free conditions and embryos exposed to 0.1% ethanol was zero. This showed that exposure to 0.1% ethanol during the first 2 days of incubation was unlikely to kill the embryo. However, this does not mean that the embryo will not die later on due to the effects of ethanol, because 23% of them already have missing body parts and 54% are demonstrating a slow growth rate.

For the embryos subjected to 1% ethanol, there was a 25% mortality rate. This showed the severity of the effect of alcohol since it is having such a great effect at a very early stage of the embryos' development. And for the embryos that did survive, all of them showed a decreased growth rate, which will radically change the development of the embryos later on and possibly cause their eventual death.



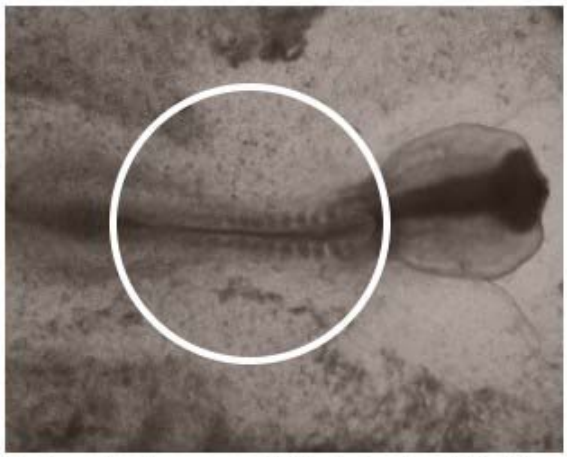
Dead embryo - head still remaining

Figure 6
Dead embryo

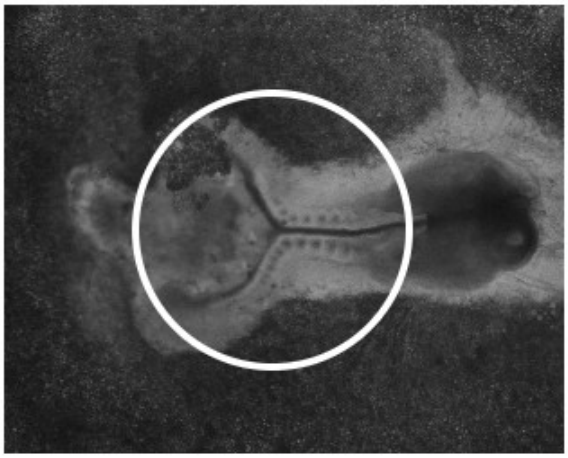
Peculiar Effects of Ethanol

Throughout the experiment, two embryos developed interesting outcomes. They were both subject to 0.1% ethanol.

Division of the Spinal chord and Somites



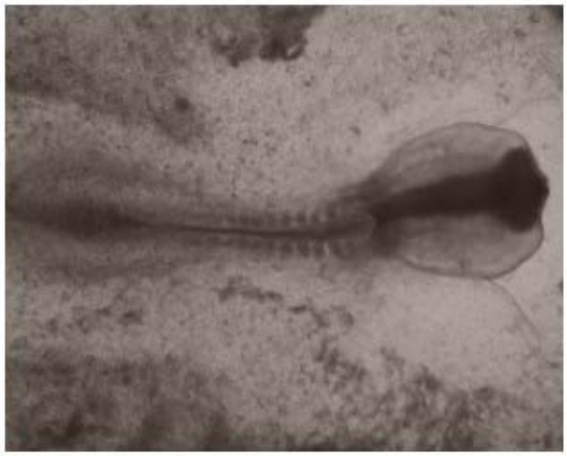
Embryo showing normal Spinal chord and Somites



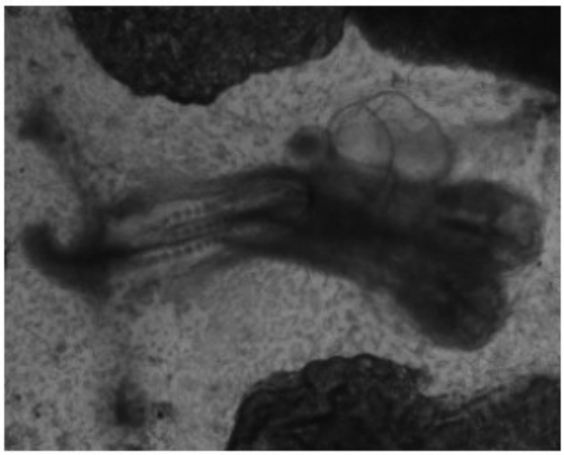
Embryo showing divided Spinal chord and Somites

Figure 7

Twins



Single embryo (normal)



Two embryos (Twins)

Figure 8

These abnormal developments may not necessarily be an effect of alcohol. It could simply be a random mutation. But neither reason can be proved, as more time would be needed to produce a greater number of replications of the experiments for any conclusions to be drawn.

DISCUSSION

Conclusion

Alcohol had an effect on the development of chicken embryos in the following ways; Table 2 showed that the presence of ethanol reduced the body length of the embryos and that as the concentration of ethanol increases, the reduction of the embryos' body length increase too. Table 3 showed some other effects of ethanol i.e. as ethanol concentration increased from 0 to 1%, the number of embryos with developing hearts decreased, there was an increase the number of embryos with missing body parts and the mortality rate increased. Both results confirm the hypothesis of ethanol having an effect on the chicken embryonic development and that the more ethanol present, the greater effect it has.

The reduction in embryo length and delayed heart development both validate the conclusion that ethanol drastically reduces the rate of embryonic development. This was one of the main findings of this investigation.

From Table 3, it can also be concluded that 1% ethanol is a lethal concentration of which cause severe damages to the embryo, with a very high chance of killing the embryo altogether.

Evaluation of Procedures

The greatest fault in this investigation was the manner in which alcohol was exposed to the embryos. In real life, the mother would probably remove some of the alcohol consumed before allowing it to have any effect on the foetus. But the embryos used in this investigation were subjected to direct exposure of alcohol (bathed in ethanol containing agar, therefore, receiving a higher concentration of alcohol than it would in real life. How much effect the mother would have on the concentration of alcohol received by the foetus would require experiments of much more complexity.

EC culture

"Remove the thick albumen covering the blastoderm by using a piece of folded tissue paper (e.g., a Kimwipe). Do this by placing the paper onto the albumen at the edge of the blastoderm and gently drawing it away from the centre. This should gradually lift the albumen away from the blastoderm." (Chapman 2001)

This part of the procedure was very difficult to do because the thick albumen was reluctant to move away from the blastoderm. Therefore, it became a time consuming process and was often not removed properly causing a problem for the vitelline membrane to attach onto the filter paper properly later on, losing the blastoderm as a result. This problem was overcome by using scissors to carefully cut through the thick albumen all the way around the blastoderm. Then use the filter paper to gently wipe the thick albumen away. However, this required lots of practise to avoid piercing through the vitelline membrane. Nevertheless, it was worthwhile because not only did this alternative method speed up the process of removing the thick albumen; it also made sure all the thick albumen was removed. Therefore, increasing the strength of the attachment of the vitelline membrane to the filter paper.

Often, there was still some excess yolk attached to the vitelline membrane after the blastoderm was placed on top of the agar in the petri-dish. Too much yolk meant it would mask the embryo. Therefore, the petri-dishes were placed under a microscope and a minute quantity of saline solution was pippetted on top of the embryo to wash away any yolk that was left. This additional procedure attained much clearer photographs of the embryos.

Adding ethanol

The timing for the addition of ethanol to the agar solution was extremely important. When it was added to the solution at the end of the standard procedure (once mixed with albumen), a wool-looking precipitation was synthesized. The precipitation was likely to be a result of the insolubility of ethanol in the existing solution, which means the ethanol added had not distributed evenly within the agar. This would cause a significant error in the results as different petri-dishes would contain different concentrations of ethanol. The problem was overcome by adding ethanol immediately after adding the deionised water to the Bacto-Agar (before the solution was mixed with albumen). This alteration minimized the production of precipitation. Therefore, reducing the error in the results.

Measurements and errors

When making the agar petri-dishes and saline solution, measurement of volumes of solutions were made. The reading of volumes produced an error of +- 0.5ml

Other measurement included measuring the length of the embryos. Obtaining the actual length of the embryos would be very difficult because they were so small that they could only be seen under a microscope. To overcome this difficulty, the embryos were photographed and printed all using the same scale. Then measured using a regular ruler. The error in measurement was +- 0.5mm.

The experiment was replicated twice and an average was calculated. The replications increased the reliability of the conclusion.

Greater accuracy of results can be achieved by improvement of equipments used.

Evaluation of Results

Evolution created naturally occurring genetic diversity in all species. This meant there were variations in the development of the chicken embryos due to genetic differences even before exposure to alcohol. Therefore, a greater number of embryos, developed under alcohol free conditions, should have been monitored to establish the embryos' natural susceptibility to abnormal development if time permitted.

Desirable modifications for improvement

If time permitted, more replications of the experiments would have been desirable as it would support and strengthen the conclusions. Also, exposing the embryos to a greater range of concentrations of ethanol would enhance the appreciation for the lethality of alcohol on the chick embryos at different quantities of consumption.

The narrow ethanol concentration range (0.1%-1%) provided limited appreciation of the variance in the effect of alcohol. 0.1% and 1% concentrations were chosen based on *Cummings* observation that: *Measurements on placentas recovered from normal births show that umbilical blood vessels constrict in the presence of alcohol concentrations as low as 0.05%.*

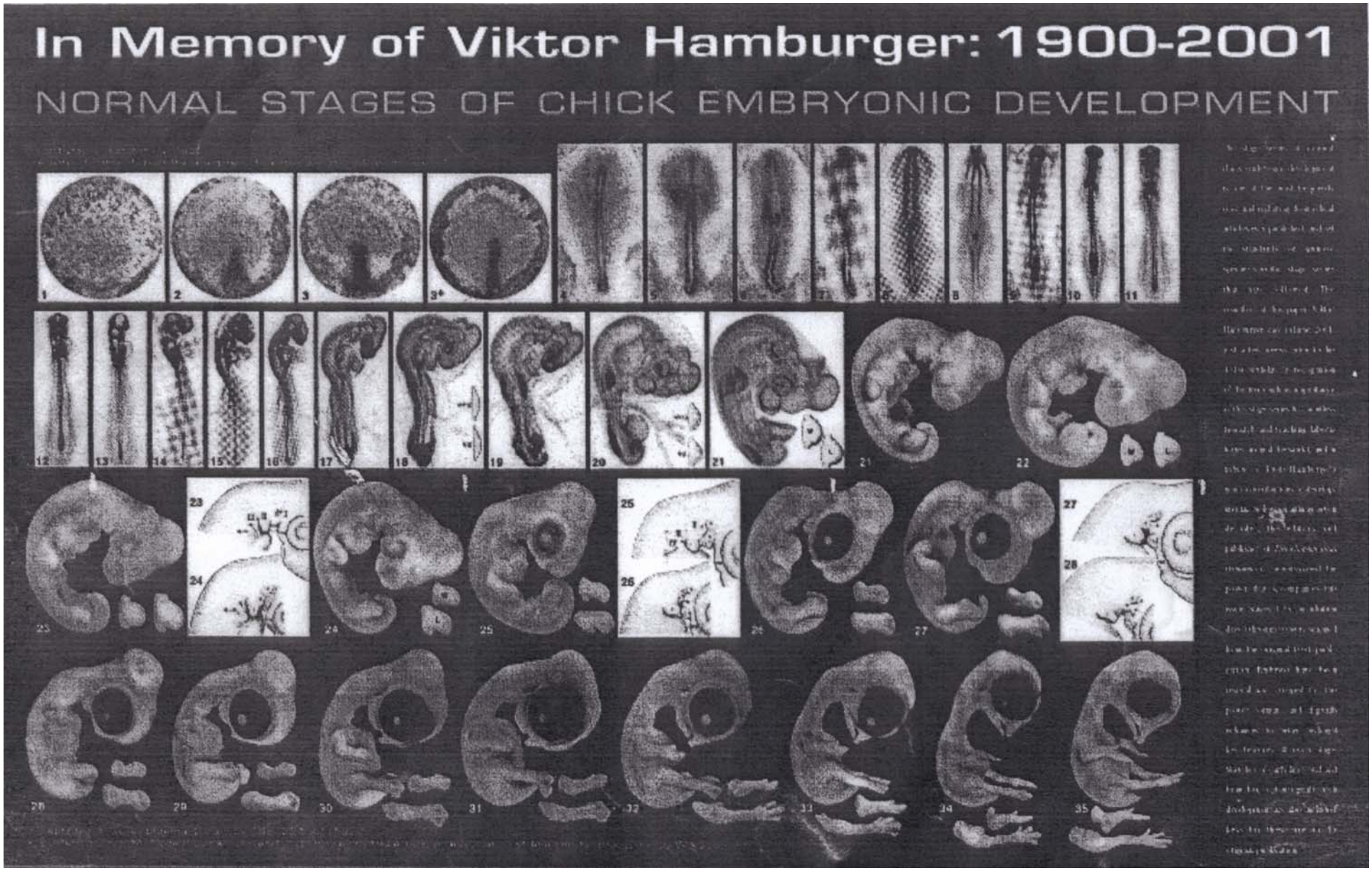
The Equivalent of two mixed drinks in one day at any time in the last 3 months of pregnancy will reduce foetus birth weight by 5%. (Cummings 1997) Monitoring the weight of the embryo would have been useful, as this would have provided a more insightful conclusion of the effects of ethanol. However this meant measuring the weight of every petri-dish with the agar and embryo before and after incubation. The very sensitive weighing equipment required to weigh embryos of only 0.003g was not available.

The rate of growth can also be determined by categorizing the stage of the embryos development. These stages are called the Viktor Hamburger Stages. Categorizing the stage of the embryo should have been used as an indication of how far developed the embryo was instead of its length. This would improve the comparison of embryonic rate of growth by discarding the errors made in body length measurements and the body length differences caused by genetic diversity.

Errors

The small errors made from measurements should not drastically affect the overall results and the conclusions drawn from them. This is due to the more significant error producing factors such as genetic diversity, which induced errors far greater than any inconsistencies in measurements.

The investigation produced the hypothesized results and succeeded in verifying certain effects of FAS. However, it would be unfair to account for the effects of alcohol totally based on the results obtained or consider the results as realistic effects. The actual fetal development of a human life cannot be mimicked and analysed based on the simple observations of chicken embryonic development. Furthermore, the complexity of biological development in animal means that much more sophisticated experiments would be required to obtain any sort of realistic conclusions. However, this investigation achieved to present a general overview for the effects of ethanol on developing embryos and convincingly demonstrated its lethality as well as the devastating consequences.


In Memory of Viktor Hamburger: 1900-2001
NORMAL STAGES OF CHICK EMBRYONIC DEVELOPMENT

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