Effects of Caffeine on Embryo Growth in Chicks

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Introduction:

The United States Food and Drug Administration began warning women about harmful effects of caffeine during pregnancy back in 1980 because of outcomes of recent studies involving effects of caffeine during pregnancy using animal models. There has been a link between high caffeine consumption and low birth weights as well as growth retardation. This becomes an even higher concern during the third trimester of pregnancy. (Fenster et al, 1991). Caffeine passes freely across the placenta once it has been absorbed through the digestive system of a pregnant woman. The half live of caffeine becomes much longer during pregnancy and the fetus does not metabolize caffeine well (Bech et al., 2007). Due to the fact that caffeine increases levels of circulating catecholamine's this can possibly lead to uteroplacental vasoconstriction and fetal hypoxia. These both may reduce fetal growth (Bech et al., 2007). Another effect of caffeine is that it increases the levels of cyclic adenosine monophosphate in cells which may influence and impair cell development, in turn having an effect of total growth. Although many factors such as these have been discovered, there have also been studies that have found no association between caffeine intake and intrauterine growth retardation (IUGR). (Vik et al., 2003).

In order to conduct a study to help determine if caffeine effects fetal growth, chick embryos will be used. The reason for using chicks, is that animal models can address one or more factors in question and help provide information which can be related to the risks in humans. (Zajac & Abel, 1992). Based on the preceding information, it is believed that injecting chicks with 0.2mg/ml caffeine solution daily will cause a decrease in the area (total growth) of the leg bud and wing bud when compared to the embryos not exposed to a chronic caffeine solution.

Materials and Methods:

Background Information:

To begin the experiment, a caffeine solution will be made by mixing 10mg of powder caffeine with 50ml of the tyrode, creating a 0.2mg/ml caffeine solution. Also the antibiotic solution will be made and ready to use. Having these solutions ready will make things easier later on. This experiment is intended to be run for approximately 10 days. During this 10-day period, the chick embryos will be kept in an incubator at 38° Celsius only removed for measuring.

Chick Embryo Explanting:

A total of 9 successfully explanted 72-hour chick embryos will be needed to allow for enough data to be collected. Before

retrieving the eggs from the incubator, be sure all materials needed are easily accessible. Explanting the eggs into Petri dishes will allow for observations to be made. It is important to keep the work area clean and sterile so as not to interfere with the chick embryos. The workspace will be disinfected with 70% EtOH solution and paper towels. All researchers will wear latex gloves and sterilize them with the 70% EtOH solution as well. Tools will be collected prior to beginning the experiment, these tools include a pair of forceps, a paper egg, and a plastic beaker incase of egg breakage during explantation. Prior to explanting the eggs are developing to the 3day stage and stored at 38° Celsius.

When an egg is removed from the incubator, it will be disinfected with the 70% EtOH solution and placed in the egg tray wide end down to air-dry. By placing it in the egg tray in this position, the yolk is allowed to rotate so the embryo faces the eggs narrow end and is away from the airspace. Once the egg is dry, it will be held with wide end up over the plastic beaker and the forceps will be used to gently crack a tiny hole in the top. This will expose the airspace and the shell will be carefully peeled away using the forceps until the hole reaches the edge of this airspace. During this step it will be very important that people do not puncture the membrane underlying the airspace. Once the hole is as smooth as possible and covering the entire airspace, the egg will then be held over a weigh boat inside a sterile Petri dish with the narrow end up. At this point nothing will come out of the egg due to air suction. In order to have the embryo slowly fall into the Petri dish without the yolk breaking, a very tiny hole will be made carefully in the narrow end of the egg, which will allow the embryo to slowly leak out of the wide end into the weigh boat. If the embryo does not leak out, a small puncture can be made in the shell membrane at the wide end to give the embryo an opening to go out. This process will be repeated until we have a total of 9 embryos in separate Petri dishes. (Pingree, 2007).

Caffeine Exposure:

To give the idea of a chronic caffeine exposure, the embryos will be injected with 0.5ml of the 0.2mg/ml caffeine solution each day the experiment is run (Crawford, 2007). The experiment will run for 10 – 14 days, given the chick embryos survive that long. We did not want to give them too high of a dose of caffeine because we do not want them to die after two days if we can prevent it. The embryos in the control group will be injected them with 0.5ml of just the tyrodes, this keeps the amount of injected substance equal across all the embryos.

Data Collecting:

I will be measuring the growth of the wing buds and limb buds on each of the embryos. To collect data I will be using a microscope in order to make it easier to measure these structures, as they are too small to measure with the naked eye. I will make measurements using the ocular units and then convert them into millimeters to more easily compare the data. A method described by Professor Morris to measure the area of the wing buds and leg buds to give more detail about the progression of development of these structures. The data will be recorded in a data table and analyzed at the end of the experiment. There will be no need to quantitate the data, as they will already be in the form of numbers. Pictures will be taken of the embryos

using the microscopes and video equipment in the ICU to visualize the growth.

Results and Conclusions:

My hypothesis states that: Injecting chicks with 0.2mg/ml caffeine solution daily will cause a decrease in the area (total growth) of the leg bud and wing bud when compared to the embryos not exposed to a chronic caffeine solution. This was based upon the literature reviewed which talked about the effects of caffeine on fetal growth. Many experiments have noted that mothers who injest lots of caffeine during pregnancy tend to have children of low birth weight, giving the idea that the fetus suffered some growth deficiencies. If my proposed hypothesis is true than I expect to see a notice able difference in the size and area of the embryos with chronic caffeine exposure and the control embryos. If caffeine truly affects the growth of an embryo than, the embryos injected with caffeine daily should present smaller limb buds than the embryos not being injected with caffeine. Hopefully data will be collected on all 9 embryos for the full 10 days, although some embryos most likely will not make it the full 10 days. If these are indeed the results gained from the data collected, then it will be possible to conclude based on these results that chronic caffeine can impact growth of the limb buds and possibly other structures of an embryo.

References:

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