Chronic hypoxia impairs embryonic growth but did not affect ventricular wall thickness and cardiomyocyte size in the broiler chicken fetus

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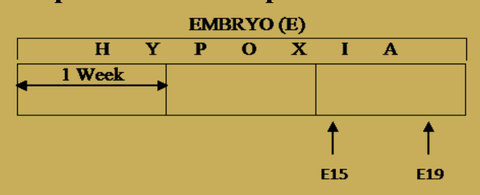
Background:

- Chronic hypoxic exposure during development can produce impact in both anatomical and physiological ontogeny.
- When exposed to hypoxic conditions chicken embryos develop relatively larger hearts than under normal conditions.
- Previous studies suggest that lower oxygen tension in the fetus is essential for normal heart formation.
- However, physiologically, hypoxia will lead to adverse effects which lead to changes in structure, function and gene expression in fetal hearts.
- Studies in chickens to understand the hemodynamic changes (mean arterial pressure, heart rate and peripheral arterial resistance) during fetal development under hypoxic conditions and transition of cardiomyocytes from hyperplasia to hypertrophy during postnatal development have been extensively made .
- But the cardiovascular changes and its effects during fetal development under hypoxic condition are poorly understood.

Objective:

This study aims at measuring ventricular wall thickness and cardiomyocyte size to understand physiological changes in structure of the heart due to chronic hypoxic exposure at 15 and 19 days of incubation age.

Experimental set up:



Experimental groups:

Treatment	Age (Days)
Normoxic (N) $21\%O_2$	15 and 19
Hypoxic (H) $14.5\%O_2$	15 and 19

- The animal model used was a domestic broiler chicken strain (Gallus gallus)

Experimental design:

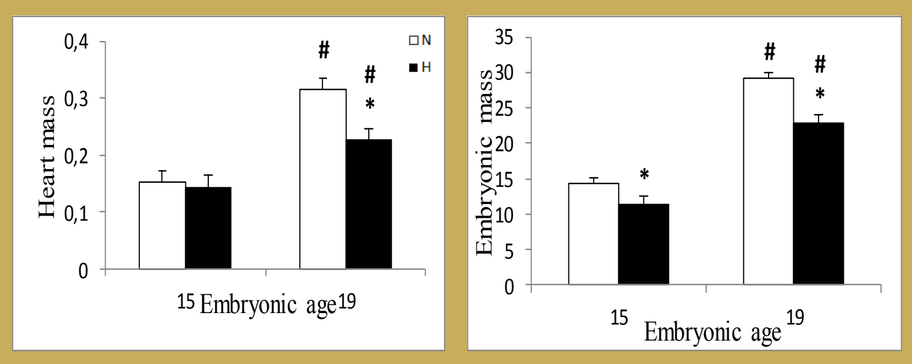
Cardiomyocyte size measurement:

- Cardiomyocytes are isolated through retrogarde perfusion technique.
- Myocyte slurry is fixed with PFA and stained with DAPI stain.
- Processed slided are viewed under flourescent microscope and cells pictures are captured with the help of QIMC camera.
- Cells are measured manually with NIS-AR elements software.

Ventricular wall thickness measurement:

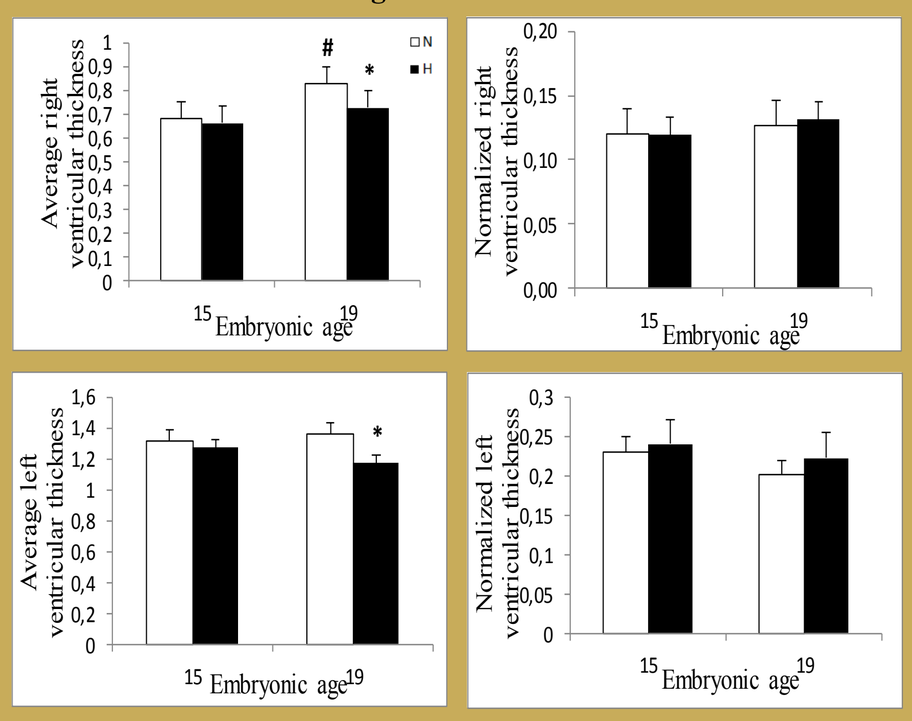
- Excised hearts were rinsed in PBS buffer and pinned to silicon plate.
- Through the two catheters inserted into pulmonary artery and aorta, PFA is flushed into the chambers and then stained with 1%menthylene blue in PFA.
- The heart is flushed with PBS and filled with embedding media (OCT gel).
- The heart is made frozen at -80°C and sections are made in cryostat.
- Sliced sections are captured with mediscope and pictures are analyzed with NIS-AR elements. Measurements are made manually and calibrated.

Result 1— Chronic hypoxia impaired embryonic and heart growth



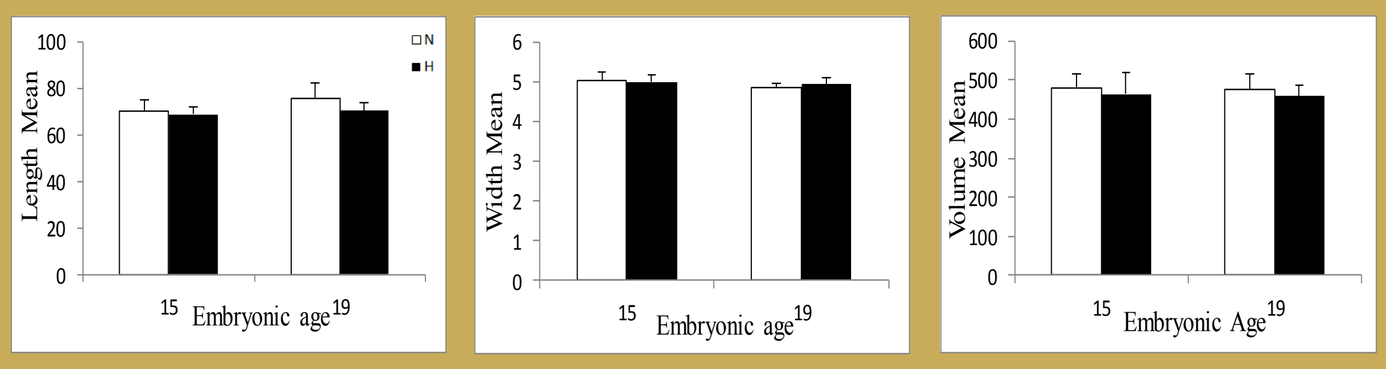
*This figure shows that hypoxic exposure impaired embryonic growth and heart growth compared to the normoxic groups. # and * symbols denote the significant difference with respect to treament and age respectively.*

Result 2— Chronic hypoxia did not affect the thickness of ventricular wall in both the incubation age



*This figure shows the average wall thickness measurements and the wall thickness normalized with respect to the mean diameter of each heart sample in both 15 and 19 day old embryos. # and * symbols denote the significant difference with respect to treament and age respectively.*

Result 3— Hypoxic exposure did not produce cardiomyocyte hypertrophy in the third stage of incubation



This figure shows the mean measurements of length width and cell volume of chicken cariomyocyte in 15 and 19 day old embryos.

Take Home Message:

Chronic hypoxia is not affecting the structure of heart in 15 & 19 day old chicken embryos!!!

Future perspects:

Does chronic hypoxia affects the strucure of heart and impose cell hypertrophy during the prenatal or perinatal stage?

Conclusions:

1. There is no change in ventricular wall thickness due to chronic hypoxia at both stages of incubation.
2. There is no cardiomyocyte hypertrophy in both 15 and 19 day old chicken embryos due to chronic hypoxia
3. There are no binucleated cells due to hypoxia during the last stage of incubation

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