CHANGES IN OXIDATIVE STRESS AND CALCIUM SIGNALLING PATHWAYS IN THE CHRONICALLY HYPOXIC FETAL HEART

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Cyanotic congenital heart disease (CHD) is associated with a greater risk of adverse outcomes following cardiac surgery (Najm et al. *J Thorac Cardiovasc Surg* **119**:515,2000). Research suggests that many cyanotic CHD patients will also have had reduced oxygen levels before birth (Sun et al. *Circulation* **131**:1313,2015). Whether exposure to cyanosis during development causes molecular changes in the heart which may affect surgical outcomes is unknown.
Pregnant sheep were exposed to normoxia (N) or hypoxia (H: 10% O_2) between days(d) 105-138 of pregnancy (term ~145 days, n=16 per group). At d138, fetal hearts were frozen for molecular analysis or mounted on a Langendorff preparation to determine function, followed by fixation for stereology.
131 proteins were differentially expressed in the left ventricle (LV) of H fetuses. Ingenuity Pathway Analysis (IPA) highlighted superoxide radical degradation as a significantly enriched canonical pathway ($p=3.84 \times 10^{-2}$) due to the upregulation of the antioxidant SOD2, a finding validated by Western blotting. Functional and stereological analyses showed that H fetuses have impaired diastolic function but maintain contractility despite thinning of the LV wall. IPA analysis in H fetuses also highlighted calcium signalling as a significantly enriched function ($p=1.84 \times 10^{-2}$), showing β-tropomyosin upregulation, which has been linked to diastolic dysfunction (Muthuchamy et al. *J Biol Chem* **270**:30593,1995) and HDAC8 downregulation, linked to compensatory maintenance of systolic function (Meraviglia et al. *Int J Mol Sci* **19:** 419, 2018).
Proteomic analysis deepens insight into molecular mechanisms underlying changes in cardiac development in the hypoxic fetus.

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