## Examination of the expression of the cardiac muscle regulatory molecules, troponin T, I and C in the sheep heart across late gestation

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During development, the fetal heart undergoes a progressive increase in the ability to produce force related to evolving function. Developmental changes in some regulatory proteins of the cardiac contractile apparatus have been shown with gestational age. The troponin I molecule (Kruger *et al.,* 2006) has been examined in relation to birth with no attention to troponin T or C, major determinants of force development. Our understanding of the developmental changes in expression of various Troponin molecules is complicated by the fact that many studies are carried out in mammalian species, particularly in rats and mice, which undergo cardiomyocyte maturation after birth whereas this occurs in late gestation in humans and sheep. The aim of this study was to determine the expression of each of the major troponin molecules, T, I and C during late gestation fetal heart development.

All procedures were approved by the University of South Australia's animal ethics committee. At 110-125 days (d) gestation, surgery was performed in 13 pregnant ewes under aseptic conditions and general anesthesia. Vascular catheters were inserted as previously described in the maternal jugular vein, the fetal femoral and carotid arteries, jugular vein, and the amniotic cavity (Morrison et al., 2007). Fetal carotid arterial blood gas samples (0.5 ml) were collected daily for the measurement of fetal blood gases. At a range of gestational ages (110-140 d; term, 150 d), ewes ($n$ = 23) were humanely killed with an overdose of sodium pentobarbitone (Lethobarb, 25 ml; 325 mg/ml) and fetuses were delivered by hysterotomy and weighed. The apex of the left ventricle was flash frozen in liquid nitrogen and stored at -80°C. The heart was reverse perfused with collagenase and protease to isolate cardiomyocytes which were fixed in 2% paraformaldehyde to allow for measurement of cardiomyocyte size and the percent of mononucleated cardiomyocytes. Western blots were performed on heart tissue using the Invitrogen NuPage Bis-Tris electrophoresis system. Primary antibodies for cardiac isoforms of Troponins C, T and I (Abcam Inc, Cambridge USA) were diluted 1:5000 and incubated on blots overnight at 4°C. Membranes were then incubated with a horse anti-mouse HRP conjugated secondary antibody at 1:2000 for 1hour (24°C), and bands visualized using ECL. Band densities were calculated using Quantity One software relative to internal controls.

Despite a decline in the percentage of mononucleated cardiomyocytes with increasing gestational age, there was no change in the expression of either troponin T or I. There was also no significant correlation between troponin T and I with either fetal weight, percent of left ventricular mononucleated cardiomyocytes (LVMC) or the size of left ventricular binucleated cardiomyocytes (LVBC). Troponin C, however, showed a 40% increase in protein expression ($p < 0.04$) after 120 d gestation. Furthermore, this increase in troponin C was positively correlated with both gestational age and fetal weight ($p < 0.04$). The percentage of mononucleated cardiomyocytes in the left ventricle was negatively correlated with troponin C protein expression.

These data show that changes in troponin C occur in late gestation tracking the timing of cardiomyocyte maturation. This may be the mechanism that supports our previous data showing little change in cardiac muscle contractility from 125 to 140 d gestation (Spencer *et al.,* 2006). Cardiomyocytes undergo binucleation after 110 d gestation in the sheep fetus (Burrell *et al.,* 2003) and this transition from mononucleated to binucleated cardiomyocytes may well be related to increased Troponin C expression and thus an increased ability to generate force.

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