

**Isolation of plasma membrane vesicles from mouse placenta at term and measurement of system A and system beta amino acid transporter activity**

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Amino acid transport across the placenta is essential for optimal fetal growth and development, with a reduced fetal provision of amino acids being implicated as a potential cause of fetal growth restriction (FGR). Understanding the aetiology of FGR due to placental insufficiency has been aided by the development of mouse models that have features of the human disease. However, to take maximal advantage of these, methods are required to study placental function in the mouse. This study aimed to develop a method to isolate plasma membrane vesicles from mouse placenta near-term and using these vesicles investigate two amino acid transporters, systems A and  $\beta$ , the activities of which are reduced in human placental microvillous plasma membrane (MVM) vesicles from pregnancies compromised by FGR. Plasma membrane vesicles were isolated from mouse placentas at embryonic day 18 by a protocol involving homogenisation, magnesium precipitation and differential centrifugation. Vesicles were enriched  $11.1 \pm 0.4$ -fold in alkaline phosphatase activity as compared to initial homogenate. Cytochemistry revealed alkaline phosphatase was localised between trophoblast layers I and II, with intense reaction product deposited on the maternal-facing plasma membrane of layer II, suggesting that vesicles were derived from this trophoblast plasma membrane. System A and system  $\beta$  activity in mouse placental vesicles, measured as  $\text{Na}^+$ -dependent uptake of  $^{14}\text{C}$ -methylaminoisobutyric acid (MeAIB) and  $^3\text{H}$ -taurine respectively, confirmed localisation of these transporters to the maternal-facing plasma membrane of layer II. Activities of systems A and  $\beta$  in mouse placental vesicles were compared to human placental MVM vesicles at initial rate. Whilst system A activity was comparable between species, system  $\beta$  activity was significantly lower in mouse. We conclude that syncytiotrophoblast layer II-derived plasma membrane vesicles can be isolated from mouse placenta and used to examine transporter function.