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Placental and fetal growth restriction, size at birth and neonatal growth alter cognitive function and behaviour in sheep in an age- and sex-specific manner☆,☆☆

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HIGHLIGHTS

- We studied cognitive effects of restricted placental and fetal growth (PR) in sheep.
- PR males at 18 and 40 weeks of age had impaired learning compared to controls.
- PR sheep performed better on reversal tasks than controls at 18 weeks of age.
- Rapid neonatal growth predicted better memory in males at 40 weeks of age.

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ABSTRACT

Intrauterine growth restriction and slow neonatal growth in humans are each associated with poorer learning, memory and cognitive flexibility in childhood and adulthood. The relative contributions of pre- and post-natal growth to cognitive outcomes are unclear, however. We therefore compared performance in learning, memory and reversal tasks using a modified Y-maze at 18 and 40 weeks of age in offspring of placentally-restricted (PR: 10 M, 13 F) and control (23 M, 17 F) ovine pregnancies. We also investigated relationships between size at birth, neonatal growth rates and cognitive outcomes. PR had limited effects on cognitive outcomes, with PR males requiring more trials to solve the initial learning task than controls ($P = 0.037$) but faster completion of reversal tasks in both sexes at 18 weeks of age. In males, neonatal growth rate correlated inversely with numbers of trials and total time required to solve memory tasks at 40 weeks of age. In females, bleat frequency in the first reversal task at 18 weeks of age correlated positively with birth weight ($r = 0.734$, $P < 0.05$) and neonatal growth rate ($r = 0.563$, $P < 0.05$). We conclude that PR induces limited effects on cognitive outcomes in sheep, with some evidence of impaired learning in males, but little effect on memory or cognitive flexibility in either sex. Rapid neonatal growth predicted improved memory task performance in males, suggesting that strategies to optimize neonatal growth may have long-term cognitive benefits but that these may be sex-specific.

1. Introduction

Intrauterine growth-restriction (IUGR) is associated with impaired neurodevelopment, with life-long consequences for cognitive function [1]. Small size at birth corrected for gestational age (SGA, size at birth below the 10th centile for gestational age) is often used as a surrogate marker of IUGR in humans when repeated measures of fetal growth are not available. Children born small for gestational age have, on average, IQ 6–11 points lower than their peers, poorer language skills, impaired spatial learning and memory, and higher incidences of behavioural and attentional problems [2–5]. These deficits have functional

Abbreviations: AGA, appropriate birth size for gestational age; BW, birth weight; CON, control; FGR, fractional growth rate; GA, gestational age; IUGR, intrauterine growth-restriction; PR, placentally-restricted; SGA, small birth size for gestational age.

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consequences, as SGA is also associated with poorer academic outcomes in children [6] and adults [7,8].

The effects of IUGR on neurodevelopmental outcomes may be ameliorated by catch-up growth in early life, suggesting an important role for post-natal growth. Catch-up growth following IUGR is common across species, including humans, where it occurs mostly during the first two months after birth [9,10]. Catch-up growth is associated with better visuomotor and problem solving skills, intelligence quotients, IQ and academic performance in SGA children, starting from 18 months and continuing into adulthood, compared to those with failure of catch-up growth [3,4,11,12]. SGA children do not always catch up in head circumference compared to peers born at an appropriate weight for their gestational age (AGA) [3,13–15], even if they are among the 86% of SGA children that catch up in height and weight [16,17]. Head circumference is an important surrogate marker for neurodevelopment, because it is strongly correlated with IQ, language, visuomotor and neurodevelopmental scores in SGA children [12,14], a relationship that strengthens with age [14].

Disentangling the influences of fetal and postnatal growth on neurodevelopmental outcomes is complicated by the common comorbidity between IUGR and preterm birth (birth before 37 completed weeks of gestation) in humans, both of which separately impair neurodevelopment and learning outcomes [4,18], with compounding effects in combination [19,20]. Human studies can also be confounded by shared prenatal and postnatal environments, and complicated by variation due to genetics and environmental factors. For example, lower socioeconomic status is associated with increased risk of SGA, a reduction in postnatal catch-up growth [21–23], and poorer cognition and executive function in both healthy [24], and SGA children [3,4,6,20]. Therefore, an animal model of fetal growth restriction, with IUGR offspring born at term, is required to further investigate the influence of fetal and neonatal growth on neurodevelopmental outcomes.

Sheep have a similar ontogeny of neurodevelopment to humans with neurogenesis, oligodendrocyte development and myelination commencing prenatally in both species [25,26]. Importantly, sheep demonstrate higher cognitive processing, including executive functions and problem solving [27,28], and learning, memory and cognitive flexibility can be tested in this species using maze tasks [28–32]. Impaired placentation, which reduces the supply of nutrients and oxygen reaching the fetus, is a major cause of IUGR in developed countries [33]. Restriction of placental growth (PR) in sheep, by surgical removal of placental attachment sites prior to pregnancy, reduces nutrient and oxygen supply and is associated with similar fetal outcomes as occurs in human IUGR, including endocrine adaptations [34–37]. PR results in delivery of full-term lambs with reductions in average birth weight of 20–31% [38,39]. PR lambs also undergo neonatal catch-up growth, with incomplete catch-up of skull width [40,41], consistent with growth patterns in IUGR infants [39–42]. This model allows effects of IUGR to be tested independent of confounders such as preterm birth and environmental differences, since all individuals share a common postnatal environment. We therefore tested the hypothesis that in adolescent and adult sheep, PR, low birth weight and slow neonatal growth each impair learning, memory and cognitive flexibility.

2. Methods

All procedures were jointly approved by the University of Adelaide Animal Ethics Committee (M-2009-145 and M-2011-055) and the SA Pathology Animal Ethics Committee (135a/09) and complied with the Australian Code of Practice for the Care and Use of Animals for Scientific Purposes [43].

2.1. Animals

Generation and management of this cohort has been described previously [39]. Briefly, placental growth and function of primiparous Merino × Border Leicester ewes was restricted by surgical removal of all but four visible endometrial placental attachment sites (caruncles) from each uterine horn [44,45] at least 10 weeks prior to timed mating to Merino rams. Control ewes were un-operated and were also included in the timed mating programme. Pregnant control (CON) and PR ewes were housed indoors from day 110 of gestation until their spontaneously-born lambs were weaned at 13 weeks of age. Groups of lambs were born at five-week intervals between July 2010 and December 2012. Ewes were fed 1 kg Rumevite pellets daily (Ridley AgriProducts, Melbourne, Australia), with ad libitum access to lucerne chaff and water. Gestational ages in days (GA), birth weight (BW) and litter sizes were recorded. After weaning, progeny were housed in outside paddocks in same sex groups of similar ages and fed 0.5 kg Rumevite pellets/sheep daily, with ad libitum access to oaten hay, pasture and water. Progeny were handled frequently from birth, with measures of weight recorded every second day from birth to 16 days of age to calculate fractional growth rate for weight [FGR, [46]], followed by weekly weighing until weaning. All animals were fed daily by an animal technician, providing frequent human contact and ensuring lambs were habituated to close contact with humans.

2.2. Learning evaluation

Maze tests were performed at 18 and 40 weeks of age as described previously for control animals [32] using a protocol modified from Erhard et al. [30] and Hernandez et al. [31]. Here we report outcomes from animals tested at 18 weeks of age and retested at 40 weeks of age; consisting of 40 control progeny (1 male and 1 female from singleton births, 22 males and 16 females from multiple births) and 23 PR progeny (5 males and 10 females from singleton births, 5 males and 3 females from multiple births).

Briefly, the test protocol consisted of 3–5 days of testing [32]. The first day commenced with a habituation task, in which sheep undertook five trials in which they exited the maze through either of the open gates at their own leisure, allowing them to habituate to human handling (guidance from handler to the start position at the start of each trial and presence of recorder behind start position), the maze itself and maze protocols (travelling from the start position when start gates were opened through the diamond maze to an open gate). The gate most frequently exited in this task was recorded as their preferred side. Time was not recorded for this task, however the majority of sheep exited the maze in under 30 s for each trial. During all behavioural testing two experimenters were involved in the protocol, which consisted of a series of five tasks, with progression to subsequent tasks requiring successful completion of earlier tasks (Supplementary Fig. 1). On day 1, sheep first completed one guided run on each side of the maze (handler guiding sheep first to closed gate and then back around diamond maze to the open gate). For subsequent tests, the recorder was positioned in a set position behind the maze entrance, with a clear view of the entire maze; and the handler moved the sheep being tested to the starting pen at the beginning of each trial, and remained out of sight during each trial. The guided runs were followed by a learning task in which the sheep were required to exit the maze only through their preferred side (Task L). On day 2, sheep first performed a memory task (Task M1) which involved repetition of task L from the previous day. This was followed by a reversal task, requiring completion of the maze with the open gate switched to the non-preferred side (Task R1). On day 3, the sheep performed a memory task (Task M2); repeating task R1 with the open gate on the non-preferred side, and then the open gate was switched back to the preferred side for the final reversal task (Task R2).

The criterion that had to be met to complete each task was three consecutive correct exits from the maze within either 6 trials (Task L) or 10 trials (Tasks M1, R1, M2 and R2), with each trial completed within three minutes, similar to previously published definitions [31,