

# An ultrasound-guided procedure to administer a label of DNA synthesis into fetal sheep

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**Abstract.** A novel technique was developed to deliver a bolus dose of a DNA label into the peritoneal cavity of fetal sheep at 85–130 days gestation. Use of markers to identify the site of injection in fetuses from litters up to quadruplets, and immunohistochemistry to detect the DNA label, 5-bromo-2'-deoxyuridine (BrdU), confirmed the procedure was successful in 85% of cases. Duration of the procedure was (mean  $\pm$  SD) 44  $\pm$  16 min, and recovery from anaesthesia was rapid and uneventful in all cases. Fetal weight was estimated with a high degree of accuracy (residual standard deviation (RSD) = 297 g and  $r^2 = 0.93$ ,  $P < 0.001$ ) and the dose of label administered (110  $\pm$  33 mg BrdU/kg fetal weight) was adequate in all cases. BrdU detected in fetal nuclei following injection into amniotic fluid highlights the need for positive identification of the injection site in timed, short-term studies, and suggests potential to further develop the technique to investigate cellular events in fetal sheep younger than 85 days of gestation. The results demonstrate that the procedure can be used to determine *in vivo* whether or not nuclei have entered the S-phase of the cell cycle.

## Introduction

Intraperitoneal injection of a DNA label has been used to measure cell cycle activity in postnatal animals (Mozdziak *et al.* 1994), and, via injection into the dam, to provide qualitative information on the timing of nuclear events in prenatal rodents (Zhang and McLennan 1995). In large animals such as sheep, fetal catheterization is used to administer solutions directly into the fetus, but requires major surgery and a prolonged recovery period before conduct of experiments. Exposure of the fetus and subsequent injection represents a further alternative, but the duration and severity of this procedure is likely to confound results of short-term labelling studies. Injection or infusion into the dam is a less stressful option, but is costly in large animals due to the large dose needed to ensure adequate labelling of fetal DNA, and evidence suggests that the label of DNA synthesis, 5-bromo-2'-deoxyuridine (BrdU) does not cross the ovine placenta (McCoard 1998). Because of these shortcomings, there have been serious limitations to the scope of experiments aimed at quantifying cell cycle activity in fetuses of large animals.

Ultrasound is routinely used to establish pregnancy status and litter size (White and Russell 1987), to measure growth of fetuses (Barbera *et al.* 1995), and to allow tissue samples to be obtained for clinical and experimental purposes (Chervenak *et al.* 1993). We hypothesized that the use of real-time ultrasound to guide a needle into the peritoneal cavity of the sheep fetus would provide a rapid and relatively

non-invasive method to administer a DNA label. We present and discuss the development of this technique.

## Materials and methods

### Experimental animals

All procedures were performed with the approval of the Cornell University Institutional Animal Care and Use Committee.

Fifty-five Finnsheep  $\times$  Dorset ewes were selected and managed as a separate flock. A vasectomized ram was maintained with the flock to identify ewes in oestrus, which were then segregated from the flock and mated to a Suffolk ram. Pregnancy status was diagnosed at about Day 40 of gestation, and litter size estimated at about Day 50 transabdominally by an experienced operator (R.M.S.) using an Aloka 210 ultrasound unit (Corometrics Clinical Systems, Wallingford, CT, USA) fitted with a 3.5-MHz or 5-MHz transducer. Thirty of 52 pregnant ewes with litters ranging from singles to quadruplets were selected from this group and moved to a controlled environment facility at approximately 50–60 days of gestation. Ewes were group fed according to average estimated requirements for energy and protein (National Research Council 1985).

### Bromodeoxyuridine injection procedure

The procedure was performed during a 62-day experimental period on fetuses of 85, 100, 115 and 130 days' gestation. One hour prior to commencement of the injection procedure, a solution of 20 mg mL<sup>-1</sup> BrdU (Sigma Chemical Company, St Louis, MO, USA) in sterile 0.9% saline was prepared. At 0800 hours on the day of slaughter, ewes were weighed, moved to the surgery room and fitted with an indwelling jugular catheter (polyvinyl chloride, 0.86 mm i.d./1.27 mm o.d., Dural Plastics and Engineering, Silverwater, NSW, Australia). Anaesthesia was induced in the ewes by intravenous (IV) infusion of 10 mg ketamine HCl per kg liveweight (LW) and maintained by continuous infusion of dilute ketamine HCl (Fort Dodge