Ultrasound in Med. & Biol., Vol. 37, No. 10, pp. 1588–1595, 2011

0301-5629/$ - see front matter

ELSEVIER

doi:10.1016/j.ultrasmedbio.2011.06.016

● *Original Contribution*

ULTRASONOGRAPHIC ASSESSMENT OF GROWTH AND ESTIMATION OF BIRTHWEIGHT IN LATE GESTATION FETAL SHEEP

David J. Carr,*† Raymond P. Aitken,* John S. Milne,* Anna L. David,† and Jacqueline M. Wallace*

*Rowett Institute of Nutrition and Health, University of Aberdeen, Bucksburn, Aberdeen, United Kingdom; and †Prenatal Cell and Gene Therapy Group, UCL Institute for Women's Health, University College London, London, United Kingdom

(*Received* 19 *February* 2011; *revised* 13 *June* 2011; *in final form* 22 *June* 2011)

Abstract—Our aim was to identify which ultrasound parameters can be most accurately measured and best predict ovine fetal weight in late gestation. Singleton pregnancies were established using embryo transfer in 32 adolescent ewes, which were subsequently overnourished to produce fetuses of variable size (1720–6260 g). Ultrasound measurements at 126–133 days gestation were compared with fetal weight/biometry at late-gestation necropsy (n = 19) or term delivery (n = 13). Abdominal circumference (AC) and renal volume (RV) correlated best with physical measurements (r = 0.78–0.83) and necropsy/birth weight (r = 0.79–0.84). Combination of AC + RV produced an estimated fetal weight equation [Log EFW = 2.115 + 0.003 AC + 0.12 RV – 0.005 RV^2] with highest adjusted R^2 (0.72) and lowest mean absolute/percentage prediction error (396–550 g/11.1%–13.2%). In conclusion, AC and RV are parameters of choice for assessment of late-gestation ovine fetal growth and can be used to estimate fetal weight with similar accuracy to human fetuses. (E-mail: davidcarr@doctors.org.uk) © **2011 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Abdominal circumference, Biometry, Renal volume, Sheep, Ultrasound.**

INTRODUCTION

Assessment of fetal growth in high risk pregnancies is an important part of modern obstetric practice. The most commonly used fetal measurements for this purpose are the biparietal diameter (BPD), head circumference (HC), abdominal circumference (AC) and femur length (FL) (Loughna et al. 2009). Observations at a single time point in gestation can be compared with population or customised standards whilst serial measurements can additionally identify abnormal fetal growth velocity. This may indicate fetal growth restriction (FGR), a condition in which the fetus fails to reach its own genetically predetermined growth potential. Biometric parameters are surrogate markers of fetal body weight and in clinical practice are often used, singly or in combination, for the point estimation of fetal weight *in utero* using one of a large range of published regression equations, albeit with considerable systematic errors of up to 25%, particularly in FGR or macrosomia (Platz and Newman 2008).

Address correspondence to: David J. Carr, Rowett Institute of Nutrition and Health, University of Aberdeen, Greenburn Road, Bucksburn, Aberdeen, AB21 9SB, UK. E-mail: davidcarr@doctors.org.uk

The pregnant sheep is a widely used model for human pregnancy (Barry and Anthony 2008) and there are several established ovine models of FGR available for translational obstetric research (Morrison 2008). The ability to identify the most perturbed pregnancies within a cohort and to monitor fetal growth following a therapeutic intervention is of significant importance to researchers using sheep models to develop future therapies for FGR. Ultrasound has previously been used to track fetal growth in the sheep using a variety of biometric parameters including the BPD, AC, FL, tibia length (TL) and abdominal diameter (Barbera et al. 1995; Galan et al. 1999; Parraguez et al. 2005). However it has not specifically been established which of these ovine ultrasound indices most accurately reflect underlying fetal size and eventual birthweight.

Overnourishing adolescent sheep dams paradoxically results in late-onset fetal and placental growth restriction of variable severity, resulting in a wide range of birth weights at term (Wallace et al. 2006). This particular model uses single sire embryo transfer to establish singleton pregnancies and thereby maximises genetic homogeneity and controls for both gestational age and fetal number, presenting a unique opportunity to study

fetal growth without many of the confounding variables of spontaneous sheep pregnancy.

The primary aim of this study was to investigate the accuracy of various ultrasound measurements in two respects: (1) their relationship with subsequent physical measurements of the fetus; and (2) their relationship with fetal body weight, for which they are ultimately proxy measures. The secondary aim was to develop an estimated fetal weight equation for the sheep.

MATERIALS AND METHODS

Experimental animals and study design

All procedures were approved by the UK Home Office under the Animals (Scientific Procedures) Act 1986 and by local ethics committee review. The ewes were part of a larger study into a potential therapy for FGR and represent control animals which were not manipulated to improve fetal growth. Animals were housed individually under natural lighting conditions at the Rowett Institute of Nutrition and Health (57°N, 2°W). Embryos were recovered from superovulated donor adult ewes (Scottish Blackface × Border Leicester) four days post-insemination using a single sire (Dorset Horn) and then synchronously transferred in singleton into the uteri of 39 recipient adolescent ewe lambs (Dorset Horn × Mule), using methods exactly as described (Wallace et al. 1997). Immediately following embryo transfer ewes were overnourished (fed *ad libitum*) to restrict placental and fetal growth and thereby produce a wide range of fetal size as previously reported (Wallace et al. 2006). Pregnancy rate was determined using ultrasound on day 45 of gestation (D45) and confirmed viable pregnancies in 32 ewes (82.1%). In late gestation a further detailed ultrasound scan was performed as described below. Nineteen animals were scanned on D126 or D127 and subsequently necropsied at 131.0 ± 0.5 days gestation (mean ± SEM). The remaining 13 animals underwent ultrasound examination on D132 or D133 and these pregnancies were allowed to continue until spontaneous birth at 140.5 ± 0.6 days gestation (term for genotype = 145 days gestation).

Ultrasound examination

All scans were carried out by a single operator accredited in advanced obstetric ultrasound (DJC) using a GE Logiq 400 CL machine with a 5.0 MHz curvilinear probe. Sheep were examined whilst standing upright in a modified milking crate and loosely restrained by head collar (Fig. 1). They were provided with hay and one-to-one attention from a second member of the research team to reduce anxiety. All fetal measurements were repeated until a minimum of 3 values in reasonable agreement with each other (coefficient of variation < 5%) had been obtained. After confirming the presence of fetal heart activity, a transverse circular view of the fetal abdomen was obtained at the level of the lowermost rib just above the umbilical cord insertion. The abdominal circumference (AC) was measured in this plane using an ellipse and the trunk (transverse abdominal) diameter (TD) determined at the same level. The transducer was then moved caudally to identify the fetal kidneys and rotated to obtain a longitudinal view for the measurement of renal length (RL). The transverse and anteroposterior renal diameters (TRD and APRD) were measured at the widest point of the kidney. The right kidney was selected for all measurements unless satisfactory views were unobtainable. The renal volume (RV) was calculated using the ellipsoid equation ($RV = RL \times TRD \times APRD \times \pi/6$) as previously described for human fetuses by Jeanty et al. (1982). The umbilical cord diameter was measured within 1cm of its insertion into the fetal abdomen. To objectively assess placental size, 10 representative placentomes were randomly selected within the gravid uterine horn and measured in two dimensions at right angles in longitudinal midsection. The cross-sectional area of each placentome was calculated and these were then added together to give a "placentome index". In addition the biparietal diameter (BPD), femur length (FL) and tibia length (TL) were measured as previously described (Barbera et al. 1995).

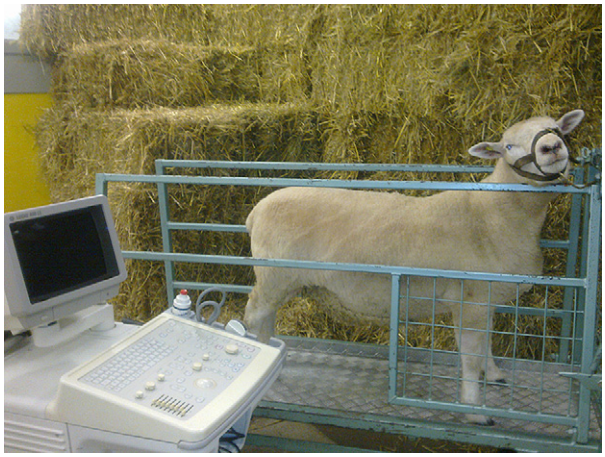

Fig. 1. Scanning the upright ewe. Photograph of the scanning set-up: the pregnant ewe stands upright in a modified milking crate and loosely restrained by head collar during transabdominal ultrasound examination.

Pregnancy outcome

Between D127 and D133, 19 ewes were killed using an overdose of intravenous pentobarbital sodium (Euthatal; Merial Animal Health Ltd., Harlow, UK). The fetus was delivered by hysterotomy and promptly killed using the same method. It was dried using a cotton towel and weighed prior to examination and full dissection. The