Impact of donor and recipient adiposity on placental and fetal growth in adolescent sheep

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Abstract

The influence of maternal obesity during oocyte development and its putative interaction with nutrient reserves at conception on pregnancy outcome were examined in an adolescent sheep model. Donor ewes were nutritionally managed to achieve contrasting adiposity (control (CD)/obese (ObD)) for 6 weeks prior to superovulation and inseminated by a non-obese sire. Morulae from 6 CD and 7 ObD were transferred in singleton into adolescent recipients of identical age but differing adiposity, classified as relatively fat or thin respectively. Thereafter, all were overnourished to promote rapid growth/adiposity (2 × 2 design, 13/14 pregnancies/group). A fifth recipient group of intermediate adiposity received embryos from another 5 CD, was offered a moderate intake to maintain adiposity throughout gestation and acted as controls for normal pregnancy outcome (optimally treated control (OTC), 19 pregnancies). Donor obesity did not influence ovulation, fertilisation or recovery rates or impact embryo morphology. Gestation length and colostrum yield were unaffected by donor or recipient adiposity and were reduced relative to OTC. Total fetal cotyledon and lamb birth weights were independent of initial donor adiposity but reduced in relatively thin vs relatively fat recipients and lower than those in the OTC group. In spite of high placental efficiency, the incidence of fetal growth restriction was greatest in the thin recipients. Thus, maternal adiposity at conception, but not pre-conception maternal obesity, modestly influences the feto-placental growth trajectory, whereas comparison with the OTC indicates that high gestational intakes to promote rapid maternal growth remain the dominant negative influence on pregnancy outcome in young adolescents. These findings inform dietary advice for pregnant adolescent girls.

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Introduction

Birth weight is a valuable aggregate of fetal nutrient supply and a robust prognosticator of health and wellbeing immediately after delivery and throughout the life-course. Our focus is the low end of the birth weight spectrum as these babies are most likely to die in infancy or experience a range of physical and development issues that can limit their life chances (Sharma *et al.* 2016). Further, low birth weight is a risk factor for the later development of a number of life-limiting diseases that drain health service resources including diabetes, stroke and cardiovascular disease, as such decreasing the proportion of babies born too early and/or too small is a pressing public health objective (WHO 2012, Hanson & Gluckman 2015).

The most consistent risk of poor outcome is when pregnancy coincides with adolescence. Accordingly both population-wide and single-centre studies in low-, middle- and high-income countries reliably report a higher risk of spontaneous miscarriage, premature delivery, low birth weight and neonatal mortality in adolescent compared with adult pregnancies

(Shrimet *et al.* 2011, Malabarey *et al.* 2012, Ganchimeg *et al.* 2013, Kozuki *et al.* 2013, Weng *et al.* 2014, Torvie *et al.* 2015). These hazards are most pronounced in very young girls when pregnancy potentially overlaps with continued or incomplete growth of the mother, setting up a competition for nutrients between the maternal body and the gravid uterus, which results in attenuated fetal growth (Frisancho *et al.* 1985, Scholl *et al.* 1994, 1997, Frisancho 1997). A similar maternal–fetal growth competition for nutrients has been partly reproduced in a highly controlled sheep model whereby overfeeding young singleton-bearing adolescents throughout pregnancy promotes high gestational weight gains and supports continued maternal growth and increased adiposity. These rapid maternal growth rates are associated with a greater incidence of miscarriage and stillbirth and robustly result in the premature delivery of low birth weight lambs compared to control-fed (slow-growing) adolescents of the same age (Wallace *et al.* 2004). In this overnourished model, defects in early placental development including lower cellular proliferation, reduced blood vessel development and

impaired secretory function progressively compromise the growth trajectory and haemodynamic function of the placenta. Consequently by the final third of gestation and regardless of nutrient excess in the mother, the small size of the placenta and associated reduction in uteroplacental blood flows and nutrient uptakes limit fetal nutrient supply (Wallace *et al.* 2006a). This leads to a slowing of fetal growth (Carr *et al.* 2012) and at parturition about 50% of these lambs are classified as prenatally growth restricted relative to the normal birth weight offspring of the optimally nourished controls (Wallace *et al.* 2004, 2006a).

In the foregoing initial sheep studies, we concentrated on varying dietary intake and hence growth status immediately after pregnancy had been established and thus the adolescents were of similar age, weight and adiposity at conception. Acknowledging that adolescent girls enter pregnancy from diverse nutritional backgrounds and with different nutrient reserves at conception, we have additionally shown that, irrespective of gestational intake and growth status, adolescents who were relatively light and thin at conception gave birth to lighter lambs than those who were heavier and fatter (Wallace *et al.* 2010). This reduction in fetal growth was again mediated by the placenta, implying a direct effect of nutrient reserves at conception on the metabolism of the dam and her early uterine environment, leading to a compromised placental growth trajectory. However, variable nutrient intakes and/or nutritional status in the peri-conception period may additionally contribute to pregnancy outcome via effects on follicle recruitment, oocyte maturation, fertilisation and early embryo development (Sinclair & Watkins 2014). As pregnancy is normally a continuum from oocyte to fetus to delivery, it is difficult to segregate the influence of nutrition at any precise stage due to potential carry over effects between sequential stages. However, in our animal model, by using assisted conception procedures, we are able to uncouple pre-, peri- and post-conception nutritional

exposures to assess their separate or interdependent influences.

Herein, we evaluate whether the nutritional status of the embryo donor ewe (relatively obese vs normal) interacts with that of the embryo recipient (relatively fat vs thin) to influence conception rate, fetal growth, pregnancy outcome and early offspring growth in adolescent animals that were overnourished throughout pregnancy. Obese vs control (normal adiposity) donor ewes were used because obesity impacts fertility with negative effects on follicle recruitment, oocyte viability, fertilisation and early embryo development reported in animal models and in humans seeking assistance to conceive via assisted reproduction technologies (ART, Gonzalez-Anover *et al.* 2011, Purcell & Moley 2011, Kumbak *et al.* 2012, Sinclair & Watkins 2014, Velazquez 2015). Moreover, in adult humans conceiving naturally, peri-conception obesity is widely associated with a plethora of pregnancy complications including hypertensive disorders, gestational diabetes, stillbirth, fetal malformations, premature delivery and derangements in fetal growth resulting in either high birth weight or relative fetal growth restriction (McDonald *et al.* 2010, Anderson *et al.* 2013, Aune *et al.* 2014, Lutsiv *et al.* 2015, Marchi *et al.* 2015). Therefore, this study tests the hypothesis that pregnancy rate and conceptus development would be most negatively disturbed in pregnancies generated from embryos of relatively obese compared with control donors, and this would be exacerbated in adolescent recipients who were relatively thin compared with those who were fatter at conception.

Materials and methods

Experimental design

All procedures were licensed under the UK Animals (Scientific Procedures) Act of 1986 and approved by the Rowett Institute's

Figure 1 Overview of experimental design.

Ethical Review Committee. The main design is a 2×2 factorial to examine the impact of embryo donor adiposity during oocyte development vs embryo recipient nutritional status at conception on pregnancy outcome at term. These adolescent ewes were subsequently overnourished throughout gestation to promote rapid maternal body growth. A fifth contemporaneous group of optimally treated control adolescents acted as a reference point for normal fetal growth (Fig. 1).

Animals: donors

Adult ewes (Border Leicester $\times$ Scottish Blackface) of equivalent age and parity (~2.5 years old and 1 previous pregnancy/lactation) destined to become potential embryo donors were selected from the Institute's flock, group housed and nutritionally manipulated by varying quantities of diet offered over a 3-month period to achieve different adiposity levels (control vs relatively obese). For the 4 weeks prior to starting superovulation protocols (6 weeks prior to embryo recovery), animals were individually housed under natural lighting conditions and offered maintenance rations of a complete diet (see below) to maintain weight and adiposity score. The latter was evaluated, based on manual palpation of lumbar spine, ribs and tail-head, by one experienced operator on a scale of 0–5, where 0 = extremely emaciated and 5 = extremely obese, according to the criteria of Russel and coworkers (Russel *et al.* 1969). The accuracy of this scoring system has been validated against whole carcass chemical analyses and is sensitive to within 0.25 score units (Wallace *et al.* 1999). On the day prior to insemination, the adiposity (mean $\pm$ S.E.M.) of potential control compared with relatively obese donors was 2.2 ± 0 units vs 3.4 ± 0.11 units, equivalent to approximately 22% and 33% body fat respectively (Russel *et al.* 1969). Donor ewes were adult rather than adolescent as their embryos are inherently more viable (Quirke & Hanrahan 1977, McMillan & McDonald 1985).

Recipients

Meanwhile two groups of adolescent ewe lambs of identical age (~7.5 months) but markedly different adiposity scores were selected from another flock (Dorset Horn $\times$ Greyface) 4 weeks prior to assisted conception procedures, destined to become embryo recipients. During this 4-week period, animals were individually offered maintenance rations of a complete diet to maintain their initial weight and thereby adiposity score. For ease of presentation, adolescents who were light and had a low adiposity score were classified as relatively thin, whereas those that were heavier and had a higher adiposity score were classified as relatively fat. Immediately prior to embryo transfer the adiposity score of these 'thin' vs 'fat' groups was 2.0 ± 0 and 2.7 ± 0.02 units equivalent to approximately 20% and 26% body fat respectively.

Embryo transfer

Using techniques described previously (Wallace *et al.* 1997), donor ewes were intrauterine inseminated on Day 0 by a single sire with optimal adiposity for breeding (Dorset Horn,

body score 3) and embryos were recovered at laparotomy on Day 4 after oestrus. Corpora lutea were counted to establish ovulation rate and embryos morphologically graded under a stereomicroscope. Embryos classified as good early morula (grade 1) and from either control or obese donors were synchronously transferred singly into the uterus of either fat or thin recipients (main study, 2×2). A contemporaneous third group of adolescents of intermediate or control adiposity (2.3 ± 0.01 units, 23% body fat) received embryos from control donors (optimally treated control (OTC) reference group).

Embryo transfers were carried out on six separate days during the mid-breeding season. Equivalent numbers of fat and thin adolescents were set-up and synchronised as potential recipients for each day (7 or 8 animals per adiposity classification per day). On the first and third day, potential embryo donors were controls, and on the second and fourth days, donors were obese. On each of these days, the aim was to transfer equal numbers of embryos from an individual donor into the two groups of recipients while taking care not to over-represent a particular donor's genetics. Thus, a maximum of eight embryos per donor were used in the study (average 5.5, range 2–8). The fifth and sixth days were reserved for the transfer of control donor embryos into the control (intermediate) adiposity recipients destined to receive a control dietary intake (OTC group). Three potential embryo donors ($2 \times$ control and $1 \times$ obese) had either regressing corpora lutea or 100% unfertilised oocytes, and these animals took no further part in the study. Similarly, a small number of potential recipients either failed to ovulate or had regressing corpora lutea when examined by laparoscope prior to embryo transfer and were discharged from further study. For the main study, 37 and 34 embryos representative of 6 control and 7 obese adult donor genetics were transferred into 71 adolescent recipients ($36 \times$ thin and $35 \times$ fat). This yielded 4 main study groups, namely control donor-thin recipient (CD-TR, $n=19$), control donor-fat recipient (CD-FR, $n=18$), obese donor-thin recipient (ObD-TR, $n=17$) and obese donor-fat recipient (ObD-FR, $n=17$). A further 26 embryos representative of a further 5 control donor ewes' genetics were transferred into the control adiposity reference group destined to become the OTC group (Fig. 1).

Nutritional management

The complete diet used throughout supplied 12 MJ metabolisable energy (ME) and 140 g crude protein per kg and was offered in two equal portions at 08:00 and 16:00 h daily (see Wallace *et al.* 2006b for full details of the diet composition and analyses). This diet was used to achieve the contrasting nutritional states in donors and recipients before conception and in recipients during pregnancy and lactation. After embryo transfer, all recipients in the main four donor $\times$ recipient adiposity groups were offered high dietary intakes to promote rapid gestational weight gain and increasing adiposity throughout gestation (overnourished; equivalent to $\sim 2 \times$ estimated ME requirements for optimum conceptus growth in ewes of this age and genotype). After a 3-day post-surgery re-alimentation period, high intakes were achieved by increasing the level of complete diet offered gradually over

a 2-week period until the level of the daily food refusal was ~15% of the total offered (equivalent to *ad libitum* intakes). The dietary level offered in the OTC group was calculated to maintain normal maternal adiposity throughout gestation (i.e. no change from initial starting adiposity) and hence meet the estimated ME requirements for optimum conceptus growth (AFRC 1993). To achieve this objective, the OTC group was fed to promote a modest maternal weight gain of ~75 g per day during the first two-thirds of gestation, followed by stepwise increases in maternal intake during the final third of gestation, calculated to meet the increasing demands of the developing fetus. During pregnancy, the level of food offered was reviewed three times weekly and adjusted, on an individual basis as and when appropriate, on the basis of daily food refusal rates (overnourished groups) and adiposity score (OTC group). After parturition, all ewes were offered the complete diet to appetite (i.e. *ad libitum*) to maximise milk availability. For the OTC dams, this was achieved stepwise over a period of approximately 10 days. Recipient ewes were weighed fortnightly, and external adiposity score was assessed approximately monthly throughout pregnancy. Ewes were also weighed ~24 h after parturition and at the end of lactation (77 days).

Conception rate, parturition management and neonatal care

Conception rate was determined by transabdominal ultrasonography at Day 50 of gestation. Pregnancy outcome was determined after spontaneous delivery, and ewes were supervised throughout the expected delivery period from Day 135 onwards (the earliest point commensurate with live birth in overnourished adolescents of this genotype). A standardised proactive regimen of neonatal care was used to prevent high neonatal mortality due to prematurity and impaired passive immunity and/or nutrient intake secondary to inadequate colostrum supply (Wallace *et al.* 1996). Lambs were dried and weighed and girth at the umbilicus was measured after delivery. The height measurement was delayed until ~12 h after birth. Ewe colostrum yield was measured before lamb suckling and within 30 min of parturition. After intravenous injection of oxytocin (Oxytocin-S 10 i.u. per ewe; Intervet Ltd, Cambridge, UK), ewes were milked by hand until all the colostrum had been removed from the udder. The colostrum was weighed, sampled for IgG analysis and then fed to the ewe's own lamb by bottle or feeding tube at a rate of 50 mL/kg body weight. In cases where the dam had insufficient colostrum, frozen pooled ewe colostrum collected previously immediately after birth (Day 145–147 of gestation) from optimally nourished adult twin-bearing ewes was substituted to ensure lamb survival. All lambs were weighed at 4 hourly intervals throughout the first 72 h of life and at 8-h intervals from 72 to 168 h. Any lamb which failed to suckle or gain weight over an 8-h period was offered supplementary colostrum (first 24 h) or ewe milk until the ewe–lamb bond and appropriate lactation were established (by 120 h after birth in all cases). The frequency of supplementary feeds per lamb was recorded. To further reduce the risk of infection, each lamb's navel was dipped in iodine at birth and at 12 h after delivery, and all lambs received intramuscular vitamin E/selenium supplementation at birth and prophylactic

antibiotics for 5 days. After the placenta (fetal cotyledons and membranes) was delivered, its weight was recorded, and then the cotyledons were dissected, counted and weighed. Lamb weight and height were measured weekly throughout the 11-week lactation. For both parameters, absolute growth rate (AGR) was linear and was recorded as the slope of the line of best fit, determined by linear regression analysis. Current FGR (CFGR) at weekly intervals was calculated as the AGR for 0–77 days divided by the value of a parameter (weight or height) at the start of each 7-day period.

Blood sampling and biochemical analysis

Blood samples were collected by jugular venepuncture approximately 3 h after the morning feed into heparinised or non-treated vacutainers. Embryo donors were sampled once 2 days prior to embryo recovery, whereas embryo recipients were sampled at ~28-day intervals from Day 0 to 135 of gestation. For embryo donors, the heparinised blood sample was centrifuged, the plasma was harvested and stored at –20°C until glucose, leptin, non-esterified fatty acids (NEFA), urea, total protein, triglycerides, total cholesterol, low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol concentrations were determined. The non-heparinised sample was collected, allowed to clot at room temperature for 2 h, wrung with a needle, refrigerated overnight and centrifuged to yield a serum sample for iron analysis. For the embryo recipients, maternal haemoglobin content was immediately measured in the heparinised blood sample in duplicate using a Radiometer OSM-3 haemoximeter (Radiometer, Copenhagen, Denmark), whilst haematocrit was determined in duplicate after blood was drawn into capillary tubes and centrifuged at room temperature at 13,000 g (Micro Haematocrit centrifuge, Hawksley and Sons Ltd, England, UK). The residual plasma was analysed as detailed previously except leptin, which was only measured in the pre-transfer and final late gestation samples. A separate heparinised blood sample was collected in late gestation (d130) to analyse plasma viscosity, and at the same stage, a further non-heparinised sample was collected for serum iron analysis. Plasma leptin was measured within a single assay in duplicate as described previously (Marie *et al.* 2001). The limit of detection was 0.4 ng/mL, and the intra-assay coefficient of variation was 7.5%. Plasma viscosity was measured at 25°C using a Coulter capillary viscometer (Cooke & Stuart 1988). All other biochemical measurements in blood or plasma were carried out using an automated clinical analyser (KONE) and kits were supplied by the manufacturer (Labmedics, Manchester, UK). Variation between duplicates was <5% in all cases. Colostrum IgG content was determined using an ovine-specific ELISA as described previously (Wallace *et al.* 2006b): intra- and inter-assay coefficients of variation were 4.2% and 6.4%.

Data analysis

All statistical comparisons were made using Minitab (version 17, State College, PA, USA). Embryo donor hormone and metabolic blood parameters, anthropometry, ovulation and embryo recovery rates were compared by Student's *t* test.

Table 1 Donor ewe anthropometry, metabolic status and fecundity at embryo recovery.

	Control donor (<i>n</i> = 11)	Obese donor (<i>n</i> = 7)	<i>P</i> value
Weight (kg)	71.3 ± 1.38	88.0 ± 1.99	<0.001
Adiposity score	2.25 ± 0.00	3.43 ± 0.10	<0.001
Plasma			
Glucose (mmol/L)	3.4 ± 0.09	3.5 ± 0.06	0.403
NEFA (mmol/L)	0.23 ± 0.034	0.11 ± 0.034	0.031
Urea (mmol/L)	7.53 ± 0.310	7.52 ± 0.250	0.959
Total protein (g/L)	80.5 ± 1.77	86.1 ± 1.41	0.041
Triglycerides (mmol/L)	0.20 ± 0.017	0.16 ± 0.031	0.262
Total cholesterol (mmol/L)	1.78 ± 0.116	1.33 ± 0.059	0.001
LDL cholesterol (mmol/L)	0.77 ± 0.088	0.40 ± 0.033	0.005
HDL cholesterol (mmol/L)	0.93 ± 0.043	0.63 ± 0.031	<0.001
Glycerol (mmol/L)	37.9 ± 6.43	23.0 ± 4.3	0.109
Leptin (ng/mL)	8.0 ± 0.71	15.9 ± 1.69	<0.001
Serum iron (µmol/L)	29.1 ± 2.09	25.4 ± 1.22	0.197
Ovulation rate (range)	21.6 ± 3.4 (9–51)	14.1 ± 0.99 (10–18)	0.110
Oocyte/embryo recovery (%)	72 ± 6.5	54 ± 13.0	0.192
Fertilisation rate (%)	82 ± 7.8	92 ± 5.8	0.367
Grade 1 embryos (%)	61 ± 11.9	73 ± 10.6	0.478

Values are mean ± S.E.M. Significant *P* values (<0.05) are highlighted in bold.

Initial recipient conception rate, lamb gender ratio, colostrum adequacy and the incidence of lambs classified as growth restricted were compared using binary logistic regression. Lambs were classified as intrauterine growth restricted (IUGR) if birth weight was <2 s.d. below the mean birth weight of lambs in the OTC group, i.e. <4195 g. For the main 2 × 2 factorial, the impact of embryo donor adiposity during oocyte development and embryo recipient nutritional status at conception (and their interaction) on maternal (recipient) anthropometry and pregnancy outcome at term was compared using two-way ANOVA (General Linear Model). Adding donor identity as a covariate did not influence any aspect of pregnancy outcome (*P* > 0.223). Comparison of these four groups with the optimally treated controls was by one-way ANOVA followed *post hoc* by Tukey's method with confidence limits set at 1% to differentiate between groups. Both approaches were used to assess the differences in embryo recipient hormone and metabolic status at the point of embryo transfer and thereafter. As embryo donor adiposity (CD vs ObD) was without significant influence, the biochemical data for recipients receiving embryos from control or obese donors were combined, presented on the basis of recipient adiposity at conception/gestational intake and further analysed by one-way ANOVA and Tukey's method as described previously. For absolute and current fractional lamb growth rates, the ANOVA model included all possible combinations of embryo donor adiposity, recipient adiposity, prenatal growth status and offspring gender. As significant effects were overwhelmingly due to prenatal growth status (IUGR or normal birth weight) and to a lesser extent gender (males vs females) the offspring growth data are presented on this basis. Correlation coefficient analyses were by Pearson's product moment test.

Results

Donor ewe anthropometry, biochemical/metabolic status and fecundity

The nutritional manipulation of embryo donors achieved the desired contrasting adiposity status for

those ewes producing transferrable grade embryos (Table 1). All ewes in the two groups of control donors had a body condition score of 2.25 and, as there were no differences (*P* > 0.215) in any of the blood parameters measured, data were combined. Relative to controls, obese donors were characterised by low circulating NEFA and lipid levels and high circulating leptin concentrations (Table 1). Donor adiposity (CD vs ObD) did not significantly influence average ovulation, fertilisation or embryo recovery rates or the number of transferrable grade embryos obtained.

Recipient ewe conception rate, anthropometry and metabolic status

For the CD-TR, CD-FR, ObD-TR and ObD-FR groups, conception rate after embryo transfer was 74, 78, 76 and 82% respectively. The recipients (*n* = 16) that failed to become pregnant had received embryos representative of 9 donor ewes' genetics (1 or 2 embryos each). Conception rate was unaffected by donor or recipient adiposity category. Conception rate in the optimally treated controls was 73% and the embryos that failed to implant (*n* = 7) were from 4 control donors (1, 2 or 3 embryos each). The nutritional manipulation of potential embryo recipients achieved contrasting weight and adiposity status maintained to the time of embryo transfer as per experimental design and is detailed for the ewes which conceived (Table 2). Animals classified as relatively fat were on average 18 kg heavier and 0.7 score units fatter (equivalent to 8.8% body fat) than the thin group at the beginning of pregnancy. For both categories, the complete diet was available *ad libitum* throughout pregnancy (i.e. overnourished) and although recipients initially categorized as thin exhibited a greater degree of weight and adiposity gain during gestation, particularly during the first third, they remained lighter and leaner at parturition than the initially fat recipients

Table 2 Changes in maternal (embryo recipient ewe) anthropometry in relation to adiposity category at conception, embryo donor adiposity and gestational dietary intake.

Embryo donor adiposity	Control				Obese		Two-way ANOVA <i>P</i> value ⁶			Control*		One-way ANOVA <i>P</i> value	
	Embryo recipient adiposity		Fat		Thin		Fat		Donor adiposity	Recipient adiposity	Interaction		Control
	Thin	Overnourished	Overnourished	Thin	Overnourished	Thin							
Embryo recipient adiposity													
Gestational Intake													
No. of recipients (pregnant)	14	14	14	13	14						19		
Recipient wt. at ET (kg)	38.2 ± 0.37 ^a	57.6 ± 0.71 ^b	38.0 ± 0.33 ^a	55.8 ± 1.01 ^b	0.141	<0.001	0.266	46.7 ± 0.31 ^c	<0.001			<0.001	
Daily live wt. gain (g/day)													
ET to day 47	311 ± 13 ^a	204 ± 11 ^b	321 ± 12 ^a	199 ± 20 ^b	0.866	<0.001	0.621	55 ± 4 ^c	<0.001			<0.001	
Day 47–90	335 ± 12 ^a	304 ± 12 ^a	334 ± 18 ^a	307 ± 21 ^a	0.970	0.077	0.907	118 ± 13 ^b	<0.001			<0.001	
Wt. 48 h after parturition (kg)	74.2 ± 1.38 ^a	84.5 ± 1.39 ^b	73.8 ± 1.23 ^a	81.7 ± 2.62 ^b	0.371	<0.001	0.502	59.5 ± 0.51 ^c	<0.001			<0.001	
Wt. change, ET to term (kg)	36.0 ± 1.27 ^a	26.9 ± 0.91 ^b	35.8 ± 1.30 ^a	25.9 ± 2.27 ^b	0.706	<0.001	0.781	12.8 ± 0.51 ^c	<0.001			<0.001	
*Recipient adiposity score													
Day 4 (ET)	2.0 ± 0.00 ^a	2.7 ± 0.02 ^b	2.0 ± 0.00 ^a	2.6 ± 0.03 ^b	0.021	<0.001	0.022	2.3 ± 0.01 ^c	<0.001			<0.001	
Day 47	2.2 ± 0.03 ^a	2.7 ± 0.02 ^b	2.3 ± 0.00 ^a	2.7 ± 0.03 ^b	0.966	<0.001	0.020	2.3 ± 0.02 ^a	<0.001			<0.001	
Day 90	2.6 ± 0.03 ^a	2.9 ± 0.03 ^b	2.7 ± 0.04 ^a	2.9 ± 0.04 ^b	0.904	<0.001	0.339	2.3 ± 0.02 ^c	<0.001			<0.001	
Day 134	2.9 ± 0.03 ^a	3.4 ± 0.06 ^b	3.0 ± 0.03 ^a	3.4 ± 0.11 ^b	0.478	<0.001	0.471	2.3 ± 0.03 ^c	<0.001			<0.001	
Adiposity change, ET to term	0.9 ± 0.03 ^{ab}	0.6 ± 0.05 ^b	1.0 ± 0.03 ^a	0.7 ± 0.11 ^b	0.286	<0.001	0.801	0.1 ± 0.02 ^c	<0.001			<0.001	

Values are mean ± s.e.m. Significant *P* values (<0.05) are highlighted in bold.

⁶Four group comparison by two-way ANOVA; *five group comparison analysed by one-way ANOVA followed *post hoc* by Tukey's method to differentiate between groups thus, within rows values with a different superscript letter differ at *P* < 0.01; ^aexternal adiposity score determined by single experienced operator.

ET, embryo transfer; Wt., weight.

(Table 2). These changes in recipient anthropometry were largely independent of initial embryo donor category. In contrast and by design, the control adiposity recipients in the OTC group had an intermediate weight and adiposity level at embryo transfer and a relatively low rate of weight gain during the first two thirds of gestation, commensurate with their lower dietary intake. Consequently, maternal adiposity score was successfully maintained throughout gestation, as required (Table 2).

At the point of embryo transfer and relative to the control recipients in the OTC group, thin recipients had higher circulating NEFA, cholesterol, glycerol and urea and lower serum iron concentrations (Table 3). In contrast, relatively fat recipients had low circulating NEFA and cholesterol and higher glucose and leptin concentrations relative to both thin and control recipient groups. Recipient adiposity did not influence haematocrit or total haemoglobin content at embryo transfer. Changes in peripheral concentrations of plasma glucose, NEFA, urea and protein throughout gestation are shown in Fig. 2. As these blood parameters were independent of donor ewe adiposity the data are presented by initial recipient adiposity category and gestational intake only. Peripheral glucose concentrations reflected gestational intake had diverged by Day 30 of gestation (*P* < 0.001) and were higher in overnourished dams, irrespective of initial adiposity category, compared with the OTC group thereafter (*P* = 0.036 to <0.001). Plasma urea followed a similar pattern with divergent concentrations evident by Day 58 and maintained thereafter (*P* < 0.001). Circulating NEFA concentrations showed a marked decrease from baseline pre-embryo transfer levels to Day 30 of gestation in response to *ad libitum* feeding in both initially thin and fat recipients and were equivalent to the control-fed control adiposity group (OTC) at this stage. Thereafter, NEFA levels were largely similar in the fat recipient and OTC groups with one exception. At Day 86, immediately prior to the start of stepwise increases in maternal feed intake to meet conceptus requirements in the OTC group, NEFA levels were elevated (*P* < 0.001). Recipients who were relatively thin vs fat initially had lower (*P* < 0.001) NEFA concentrations from Day 58 to 133 of gestation reflecting their greater live weight gain and relative increment in adiposity score. Total plasma protein concentrations broadly reflected gestational intake and were greater (*P* < 0.001) in overnourished compared with control-fed OTC dams at Days 30, 58 and 86 of pregnancy. By late pregnancy, both LDL and HDL cholesterol concentrations also differentially reflected gestational intake, and glycerol levels were greatest in the overnourished groups who were relatively fat at conception (Table 3). In all three groups, plasma triglycerides increased between the start and the end of pregnancy to a similar degree, whereas total haemoglobin and haematocrit decreased over the same period with the largest differential observed in the OTC group. Plasma leptin concentrations did

Table 3 Recipient ewe biochemical status immediately prior to embryo transfer and in late gestation.

	Recipient adiposity at conception			One way ANOVA <i>P</i> value
	Control (<i>n</i> = 19)	*Thin (<i>n</i> = 27)	*Fat (<i>n</i> = 28)	
Gestational intake	Control	Overnourished	Overnourished	
Pre-embryo transfer				
Plasma				
Glucose (mmol/L)	4.0 ± 0.07 ^a	4.0 ± 0.05 ^a	4.2 ± 0.06 ^b	0.003
NEFA (mmol/L)	0.27 ± 0.029 ^a	0.49 ± 0.038 ^b	0.09 ± 0.013 ^c	<0.001
Urea (mmol/L)	7.1 ± 0.22 ^a	8.2 ± 0.21 ^b	7.3 ± 0.19 ^a	<0.001
Total protein (g/L)	74.3 ± 1.13	75.0 ± 0.69	72.3 ± 0.88	0.074
Triglycerides (mmol/L)	0.20 ± 0.012	0.18 ± 0.010	0.17 ± 0.012	0.233
Total cholesterol (mmol/L)	1.40 ± 0.042 ^a	1.67 ± 0.048 ^b	1.07 ± 0.035 ^c	<0.001
LDL cholesterol (mmol/L)	0.43 ± 0.027 ^a	0.51 ± 0.022 ^b	0.27 ± 0.017 ^c	<0.001
HDL cholesterol (mmol/L)	0.85 ± 0.031 ^a	1.00 ± 0.036 ^b	0.64 ± 0.022 ^c	<0.001
Glycerol (mmol/L)	35.9 ± 3.66 ^a	54.9 ± 3.90 ^b	26.5 ± 1.82 ^a	<0.001
Leptin (ng/mL)	4.8 ± 0.26 ^a	3.9 ± 0.21 ^a	10.5 ± 0.51 ^b	<0.001
Serum iron (µmol/L)	33.1 ± 1.09 ^a	28.3 ± 0.87 ^b	31.1 ± 0.68 ^a	0.001
Hb	14.3 ± 0.22	13.8 ± 0.19	13.8 ± 0.12	0.070
Hct	47.3 ± 1.25	47.1 ± 0.88	47.0 ± 0.55	0.973
Late gestation (day 130–133)				
Plasma				
[†] Triglycerides (mmol/L)	0.35 ± 0.016	0.30 ± 0.024	0.30 ± 0.028	0.386
Total cholesterol (mmol/L)	1.58 ± 0.041	1.50 ± 0.048	1.54 ± 0.044	0.506
LDL cholesterol (mmol/L)	0.45 ± 0.023 ^a	0.55 ± 0.031 ^{ab}	0.57 ± 0.033 ^b	0.021
HDL cholesterol (mmol/L)	0.91 ± 0.021 ^a	0.75 ± 0.021 ^b	0.78 ± 0.018 ^b	<0.001
Glycerol (mmol/L)	39.9 ± 2.24 ^a	34.4 ± 2.56 ^a	57.4 ± 4.17 ^b	<0.001
Leptin (ng/mL)	5.3 ± 0.41 ^a	17.0 ± 1.23 ^b	19.7 ± 1.27 ^b	<0.001
Serum iron (µmol/L)	27.8 ± 1.46	28.9 ± 1.25	30.4 ± 0.71	0.290
[†] Hb (g/dL)	11.3 ± 0.30 ^a	12.1 ± 0.21 ^{ab}	12.3 ± 0.25 ^b	0.022
[†] Hct (%)	36.3 ± 1.07	38.9 ± 0.69	39.5 ± 0.96	0.055
Plasma viscosity (mPa)	1.405 ± 0.0143 ^a	1.468 ± 0.0109 ^b	1.445 ± 0.0099 ^{ab}	0.002

Values are mean ± S.E.M. Significant *P* values (<0.05) are highlighted in bold.

*As embryo donor adiposity was without significant influence the biochemical data for recipients receiving embryos from control or obese donors was combined. Mean values within a row with unlike superscripts letters are significantly different, *P* < 0.05. [†]Change between pre-embryo transfer and late gestation concentration in same direction in all groups, *P* < 0.001.

not change between embryo transfer and late gestation in the OTC group in keeping with the successful maintenance of adiposity score. In contrast, plasma leptin increased in overnourished dams with the greatest differential evident in recipients who were relatively thin at conception, again commensurate with their relatively higher increase in adiposity. At late gestation, plasma viscosity reflected initial recipient adiposity category and gestational intake and was lowest in the OTC group.

Pregnancy outcome and supplementary feeding

Pregnancy outcome data after spontaneous vaginal delivery at term are presented in Table 4. Relative to the OTC group, gestation length was shorter in the four overnourished groups but independent of both donor and recipient adiposity. Irrespective of treatment group, average live weight gain during the first two-thirds of gestation was negatively correlated with gestation length ($r = -0.636$, $P < 0.001$, $n = 74$). Gestation length was positively associated with birth weight for the study population as a whole ($r = 0.725$, $P < 0.001$) and within the overnourished ($r = 0.621$, $P < 0.001$, $n = 55$) and OTC groups separately ($r = 0.548$, $P = 0.015$, $n = 19$). Average lamb birth weight and height were independent of donor adiposity but reduced in initially thin vs fat recipients

and lower than those in the OTC group. The differential in birth weight remained after adjusting individual birth weights to a theoretical standard 146 days of gestation. The proportion of lambs classified as IUGR was also independent of initial donor adiposity and was greater in recipients who were relatively thin vs fat at conception (70% and 36% respectively, compared with 5% in the OTC group). Similarly, the ratio of maternal weight change across gestation to birth weight was greater in thin than in fat recipients and both were higher than those in the OTC group. Total fetal cotyledon weights were also independent of donor adiposity but reduced in relatively thin vs fat recipient groups (77 vs 103 g) and markedly lower than those in the OTC group (158 g). The converse was true for placental efficiency as inferred from fetal:cotyledon weight, with thin > fat > OTC groups. The individual relationships between fetal cotyledon weight and lamb birth weight are shown in Fig. 3 with the strongest associations observed in the overnourished groups.

Nutrient partitioning to the mammary gland was also influenced by gestational intake. Colostrum yield at parturition was variable within all groups, but nevertheless positive relationships with fetal cotyledon weight and lamb birth weight were evident for the population as a whole ($r = 0.523$ and 0.556

Figure 2 Peripheral plasma glucose (A), NEFA (B), urea (C) and protein (D) concentrations in samples collected at ~monthly intervals throughout pregnancy in adolescent ewes whose adiposity status varied at conception. All ewes received a single embryo and those classified as relatively fat (solid circle) or thin (solid square) at conception were overnourished throughout gestation to promote maternal growth/adiposity. Ewes with intermediate (control) adiposity at conception received a control ration to maintain initial adiposity and acted as optimally treated controls (solid triangle). Data comparison at each stage of gestation by one-way ANOVA followed *post hoc* by Tukey's method to differentiate between groups. Mean ($\pm$ S.E.M.) values are significantly different between control, and both fat and thin overnourished groups, * $P < 0.05$, *** $P < 0.001$. Mean values are significantly different between control and fat overnourished group, $^{\dagger}P < 0.001$. Mean values are significantly different between thin and fat overnourished groups and between thin and control, $^{\vee}P < 0.001$.

respectively, $P < 0.001$). The OTC dams had a greater colostrum volume than overnourished dams and within the latter category yield was independent of donor and recipient adiposity (Table 4). IgG concentration was not influenced by donor or recipient adiposity or gestational intake but the greater yield in the OTC group meant that total IgG content was the highest in this group. Forty-five dams studied (60%) were deemed to have insufficient colostrum to meet the initial minimum requirement of 50 mL/kg body weight, and their offspring were all supplemented with frozen banked colostrum. Unsurprisingly, 29 lambs required further supplementation with colostrum/milk until an appropriate lactation and ewe–lamb bond were established. Both the number of lambs requiring supplementation and the frequency of supplementary feeds were independent of donor adiposity, recipient adiposity and gestational intake (Table 4).

Neonatal viability and lamb growth rate

Two IUGR male lambs (birth weights 2.15 and 1.92 kg, born at 137 and 138 days gestation respectively) died in the early neonatal period. These individuals were both

derived from different obese donors and were gestated by an initially thin and fat recipient respectively.

The absolute and current fractional growth rates of all viable lambs were documented until weaning at 11 weeks of age and were independent of both donor and recipient ewe adiposity. In contrast, prenatal growth status and to a much lesser extent, gender influenced growth (Fig. 4). Both absolute and current fractional growth rates (weight and height) of non-IUGR or normal birth weight lambs born to overnourished dams were equivalent to those of OTC offspring throughout the period of lactation. In contrast, prenatally growth-restricted (IUGR) lambs remained smaller at all time points ($P < 0.001$) in spite of exhibiting higher fractional growth between each week of measurement ($P < 0.01$). Irrespective of gestational intake and birth weight category, males were progressively heavier and taller than females from Day 42 ($P = 0.044$ and $P = 0.043$ respectively) until Day 77 (both $P = 0.003$).

Discussion

Herein, we used assisted conception procedures to examine the impact of maternal obesity during oocyte development and its putative interaction with nutrient reserves at conception on pregnancy outcome in young adolescent sheep. Embryo recipient nutritional status at conception, but not pre-conception donor ewe obesity, modestly influenced the fetoplacental growth trajectory while comparison with the optimally treated control group indicated that high gestational intakes to promote rapid maternal growth remain the dominant negative influence on pregnancy outcome in young adolescents.

Donor ewe adiposity

Notwithstanding achieving an 11% differential in adiposity and marked differences in circulating plasma leptin and lipid concentrations prior to embryo recovery, we found no evidence of a negative impact of relative maternal obesity (~33% body fat) on ovulation, fertilisation or embryo recovery rates. This was somewhat unexpected as we have previously recorded a 48% reduction in ovulation rate after superovulation in long-term obese compared with control ewes of the same age and genotype (McConnell *et al.* 2004), whereas in beef and dairy cattle, there is an inverse relationship between adiposity and ovarian response (Velazquez 2015). Furthermore, exposure to *ad libitum* dietary intakes for 3 weeks prior to embryo recovery has been associated with a lower superovulation response both in terms of number of adult ewes ovulating and ovulation rate per ewe (Lozano *et al.* 2003), whereas obese women undergoing ART required a higher follicle-stimulating hormone (FSH) start dose to produce fewer oocytes (Zander-Fox *et al.* 2012). There is a large body of evidence connecting peri-conception diet

Table 4 Ovine pregnancy outcome and nutrient partitioning in relation to embryo recipient adiposity category at conception, embryo donor adiposity and gestational dietary intake.

Embryo donor adiposity	Control				Obese				Two-way ANOVA <i>P</i> value [§]			Control*	One-way ANOVA <i>P</i> value
	Thin		Fat		Thin		Fat		Donor adiposity	Recipient adiposity	Interaction		
Embryo recipient adiposity	Overnourished		Overnourished		Overnourished		Overnourished				Control		Control
Gestational Intake	14		14		13		14				19		
No. of recipients (pregnant)	14		14		13		14				19		
Gestation length (days)	140.7 ± 0.53 ^a		141.2 ± 0.54 ^a		139.4 ± 0.59 ^a		140.6 ± 0.35 ^a		0.060	0.106	0.506	144.0 ± 0.35 ^b	<0.001
Birth wt. (g)	3634 ± 292 ^a		4499 ± 337 ^{ab}		3802 ± 357 ^a		4259 ± 315 ^{ab}		0.912	0.047	0.533	5509 ± 151 ^b	<0.001
Adjusted birth wt. (g)	3875 ± 296 ^a		4764 ± 341 ^{ab}		4106 ± 368 ^a		4564 ± 329 ^{ab}		0.964	0.049	0.520	5651 ± 144 ^b	<0.001
Lamb girth at umbilicus (cm)	35.0 ± 1.17		37.7 ± 1.19		35.9 ± 1.51		37.4 ± 1.31		0.785	0.119	0.638	39.7 ± 0.61	0.033
Lamb height at shoulder (cm)	33.3 ± 1.09 ^a		36.5 ± 1.01 ^{ab}		34.7 ± 1.24 ^{ab}		36.3 ± 1.05 ^{ab}		0.577	0.037	0.484	38.8 ± 0.48 ^b	0.001
Male:female	8:6		8:6		3:10		6:8		0.071	0.155	na	11:8	0.262
*Proportion IUGR	9 of 14 ^{ab}		5 of 14 ^a		10 of 13 ^b		5 of 14 ^a		0.623	0.009	na	1 of 19 ^c	<0.001
Fetal cotyledon wt. (g)	75 ± 9.1 ^a		107 ± 11.0 ^a		78 ± 10.6 ^a		99 ± 11.7 ^a		0.799	0.017	0.620	158 ± 8.2 ^b	<0.001
Fetal:cotyledon wt.	52 ± 3.3 ^a		44 ± 2.4 ^{ab}		51 ± 2.6 ^a		47 ± 3.0 ^{ab}		0.786	0.036	0.491	36 ± 1.5 ^b	<0.001
Maternal wt. at ET: birth wt.	11.5 ± 0.94 ^{ab}		14.2 ± 1.58 ^a		11.2 ± 1.13 ^{ab}		14.6 ± 1.72 ^a		0.971	0.029	0.799	8.6 ± 0.26 ^b	0.001
Maternal wt. change (ET to term): birth wt.	10.6 ± 0.82 ^a		6.7 ± 0.82 ^a		10.6 ± 1.22 ^a		7.1 ± 1.19 ^a		0.857	0.001	0.850	2.3 ± 0.11 ^b	<0.001
Colostrum yield (mL)	113 ± 26.2 ^a		156 ± 34.9 ^a		115 ± 29.4 ^a		145 ± 31.8 ^a		0.881	0.244	0.839	366 ± 51.5 ^b	<0.001
Colostrum IgG concentration (mg/mL)	79 ± 13.4		137 ± 31.8		87 ± 14.8		56 ± 5.1		0.083	0.521	0.037	88 ± 8.1	0.032
Total colostrum IgG (g)	7.9 ± 1.82		23.0 ± 11.3		13.4 ± 2.90		8.5 ± 1.73		0.502	0.449	0.140	30.5 ± 3.57	0.019
No. with inadequate initial colostrum volume	13 of 14 ^a		9 of 14 ^a		11 of 13 ^a		8 of 13 ^a		0.883	0.056	0.620	4 of 19 ^b	<0.001
No. of lambs requiring supplementary feeding	10 of 14		5 of 14		5 of 12		5 of 13		0.883	0.055	0.231	4 of 19	0.064
For supplemented lambs – no. of feeds per first 120 h (range)	5.5 ± 1.54 (1–15)		6.8 ± 2.96 (1–15)		5.6 ± 2.42 (1–15)		4.8 ± 2.33 (1–11)		0.913	0.679	0.647	2.3 ± 0.75 (1–4)	0.747
Maternal wt. change (kg), delivery to weaning	–0.4 ± 1.29 ^a		–4.4 ± 0.83 ^a		–1.5 ± 0.60 ^a		–2.9 ± 1.02 ^a		0.827	0.009	0.193	8.3 ± 0.81 ^b	<0.001
*Maternal adiposity change, delivery to weaning	–0.2 ± 0.06 ^a		–0.6 ± 0.07 ^b		–0.3 ± 0.05 ^{ab}		–0.5 ± 0.12 ^{ab}		0.872	0.001	0.273	0.2 ± 0.04 ^c	<0.001

Values are mean ± s.e.m. Significant *P* values (<0.05) are highlighted in bold.

[§]Four group comparison by two-way ANOVA; *five group comparison analysed by one-way ANOVA followed *post-hoc* by Tukey's method to differentiate between groups thus, within rows values with a different superscript letter differ at *P* < 0.01; ^aexternal adiposity score determined by single experienced operator; ^blambs were classified as intrauterine growth restricted (IUGR) if birth weight was <2 s.d. below the mean birth weight of the optimally nourished control group, i.e. <4195 g; ^cadjusted birth wt. (146 days gestation) = weight at birth/1.01305 per day of gestation. Incidence of IUGR, male sex and colostrum adequacy was compared by binary logistic regression.

ET, embryo transfer; Wt., weight.

Figure 3 Association between total fetal cotyledon weight and lamb birthweight at term in relation to maternal adiposity status at conception and gestational intake thereafter. The adolescent dams were either relatively fat (solid circle; $r=0.878$, $P<0.001$) or thin (solid square; $r=0.888$, $P<0.001$) at conception and overnourished throughout gestation to promote maternal growth/adiposity or were of intermediate adiposity at conception, nourished to maintain initial adiposity and acted as optimally treated controls (solid triangle; $r=0.592$, $P=0.009$).

and/or nutritional status with embryo quality. When the approach is similar to that used here (namely superovulation with FSH, intrauterine insemination and embryo recovery at Day 4 or 6 of the cycle), there is little impact of either acute ($1.5\times$ maintenance for 3 weeks) or prolonged ($1.8\times$ maintenance for 4 months) elevations in adult donor ewe intake on the developmental stage or transferrable quality of the embryo (Kakar *et al.* 2005,

Rattanatrak *et al.* 2010). In contrast, there is evidence that when oocytes are either fertilised and matured *in vitro*, or fertilised *in vivo* and cultured *in vitro*, overfeeding and/or obesity in the donors has a negative effect on fertilisation rate and on the rate of morula/blastocyst development (adult sheep, Grazul-Bilska *et al.* 2012; late adolescent mouse, Finger *et al.* 2015, Wu *et al.* 2015; young cattle, Adamiak *et al.* 2005). In the present study, any similar delay in embryo development *in vivo* would have resulted in lower embryo recovery rates and/or morulae with a low cell count for stage (and hence deemed non-transferrable), but we found no evidence that this was the case. Thus, either the nutritional micro-environment surrounding the oocyte in the follicle and the early embryo in the oviduct is largely independent of the donor's nutritional and hormonal status or the oocyte/embryo is relatively insensitive to local changes in nutrients at this early stage.

In the present study, only grade-1 morulae, correct for stage, were transferred in singleton to the embryo recipients. This is the only viable approach when recovering embryos at a defined time point for immediate transfer into synchronous recipients because delayed development cannot be accurately differentiated from poor quality. Here, we evaluated the putative interaction between donor and recipient adiposity for the first time. Contrary to our hypothesis, we found that the nutritional status of the embryo donor did not influence pregnancy rate or conceptus growth in adolescents subsequently overnourished to promote rapid gestational weight gains and continued maternal body growth. Few of the aforementioned ruminant studies have documented the impact of relative maternal obesity beyond the maternal recognition of pregnancy stage. Exceptionally in the study by Rattanatrak and coworkers (Rattanatrak *et al.* 2010), embryos from obese

Figure 4 Absolute changes in postnatal weight and height determined weekly (A, D), and current fractional growth rate (CFGR) during weekly periods from birth until weaning (B, E) in intrauterine growth-restricted (IUGR: striped grey bar/square; $n=27$) and non-IUGR (solid black bar/square; $n=26$) lambs from overnourished adolescent dams and in normal birth weight lambs from optimally treated controls (solid grey bar/square; $n=18$), with males and females combined. Data comparison at each age by one-way ANOVA followed *post hoc* by Tukey's method to differentiate between groups. Mean values are significantly different between IUGR compared with non-IUGR and normal birth weight lambs at all ages. For A and D, $P<0.001$ and for B and E, $P<0.01$. Changes in weight and height (C, F) in males (solid black circle; $n=34$) and females (closed white circle; $n=38$), irrespective of birth weight classification and gestational intake, $*P<0.05$, $**P<0.01$.

or control ewes were transferred singly into non-obese adult recipients. Although recipient conception rate and gestational weight gain were not expressly reported, the authors indicate that peri-conception obesity did not influence birth weight. Studies in mice are somewhat contradictory: when oocytes harvested from the Blobby mouse strain were *in vitro* fertilised and blastocysts were transferred into normal-weight recipients, the resulting fetuses were heavier than controls at E14.5 (Wu *et al.* 2015). In contrast, when the contribution of diet-induced obesity in both parents was assessed after natural mating or embryo transfer into normal weight recipients, conception rate was unaffected, but fetal and placental weights were reduced at E19, independent of *in utero* diet exposure (McPherson *et al.* 2015). However, these mouse studies (conducted in late adolescence) require groups of embryos ($n=5$ or 6) to be transferred together to ensure conception in this litter-bearing species, and this somewhat confounds the assessment of feto-placental growth, arguably not accurately replicating the human condition. Women undergoing ART may help inform the debate but, even although obese adult women undergoing IVF with autologous oocytes are variously linked with reduced fertilisation rates, lower embryo quality, increased miscarriage rates, fewer clinical intrauterine pregnancies and live births (Luke *et al.* 2011, Rittenberg *et al.* 2011, Shah *et al.* 2011, Provost *et al.* 2016), there is a relative lack of information on other key aspects of pregnancy outcome. A single study involving 739 embryo transfers has reported a 70–200 g increase in singleton term fetal weight in obese compared with normal BMI women (Zander-Fox *et al.* 2012) and, in a larger population assessment of singleton pregnancies, obese women who used ART to conceive ($n=338$) had a two-fold higher risk of early preterm birth (<34 weeks gestation) than those who conceived naturally ($n=105,650$, Sauber-Schatz *et al.* 2012). In both of these studies, however, it is not possible to separate effects of pre-pregnancy BMI from gestational intake thereafter.

Recipient ewe adiposity

Independent of donor ewe adiposity, adolescent recipients who were relatively thin at conception and who were subsequently overnourished to promote continued maternal growth throughout pregnancy delivered lambs that were on average 665 g lighter than recipients who were fat at conception. This is strikingly similar to the differential in birth weight attributed to recipient adiposity previously (555 g) when donor ewe nutrition was not varied and equated to the control donor group used here (Wallace *et al.* 2010). Together, our present and previous studies suggest that adiposity at conception is an important consideration in predicting pregnancy outcome in still-growing adolescents (see below). The difference in estimated body fat between recipient groups was quite small (8.8%), but nevertheless at the

point of embryo transfer, those classified as relatively thin had lower peripheral nutrient and metabolic hormone concentrations (glucose, iron and leptin), whereas their high lipid levels were indicative of active catabolism. Nonetheless, these small but significant differences in maternal nutrient status at conception were sufficient to differentially impact placental growth and hence fetal nutrient supply, leading to a higher incidence of IUGR in the initially thin vs fat groups. This occurred in spite of presumed placental adaptation and greater trans-placental nutrient transport efficiency, as inferred from a higher fetal: placental weight ratio in the thin group. A similar relationship between nutrient status and placental growth is inferred in humans, irrespective of age, because placental weight rises with increasing maternal BMI at conception from underweight through to morbidly obese categories (Wallace *et al.* 2012). In infertile women the use of donor oocytes is now common and while the impact of donor vs recipient BMI on pregnancy, miscarriage and live-births has been related (Jungheim *et al.* 2013, Rubio *et al.* 2015), potential effects on gestation length, placental size and birth weight have not been reported.

Gestational intake

In the present study, the slow-growing age-matched contemporaneous control group was included as a reference point for normal fetal growth. The nutritional management of this group, in terms of donor and recipient adiposity and gestational intake, replicated our standard approach and comparison with the overnourished groups, irrespective of initial recipient adiposity, serves to emphasise that high gestational intakes to promote rapid maternal growth remain the most dominant negative influence on pregnancy outcome in young adolescent sheep. This was true with respect to premature delivery, feto-placental growth and nutrient partitioning to the mammary gland, all of which confirmed prior studies with respect to the magnitude of effects (Wallace 2011) and was also evident in the differences in maternal biochemical/metabolic status during gestation and at the late gestation time point. High circulating glucose and urea (and low NEFA) concentrations are commensurate with the oversupply of nutrients facilitating a highly anabolic state, rapid growth and increased adiposity in overnourished dams. This rapid maternal growth has previously been linked to depletion of liver iron stores during the first two thirds of gestation, and with a failure of the normal blood volume expansion of pregnancy between mid and late gestation (Luther *et al.* 2010). Here, compared with the OTC group, the relative preservation of haematocrit, haemoglobin and plasma protein concentrations between the point of embryo transfer and late gestation in overnourished dams implies a similar scenario. In support, blood viscosity was highest in the overnourished dams that were relatively thin at conception, and this is likely to play a role in

the attenuation of uteroplacental blood flows and fetal nutrient supply characteristic of this animal model.

Postnatal growth

Although fetal growth was constrained by both recipient adiposity at conception and gestational intake in the current study, the degree of compromise at birth was variable. Accordingly, it was prenatal growth category and to a lesser extent gender which had the most marked influence on early postnatal growth. Fractional growth rates were high in IUGR lambs, and they remained smaller at weaning, but the long-term implications beyond this life-stage were not determined. Others have reported programmed changes in later adiposity and metabolism in prepubertal and adult ovine offspring after peri-conception obesity in adult ewes, but these effects were independent of changes in birth weight (Long *et al.* 2010, Rattanaray *et al.* 2010).

Implications for human adolescents

No animal model completely replicates human pregnancy, but nevertheless, the outcome of the current ovine study has implications for public health. For young adolescent girls, it seems that both nutrient reserves at conception and dietary intake thereafter are likely to be potent determinants of fetal growth particularly if maternal growth *per se* is ongoing or incomplete. Where early marriage soon after menarche and pregnancy during young adolescent life is the cultural norm, girls with a low BMI should be encouraged to gain weight and achieve a normal BMI and hence adequate nutrient status before pregnancy. Thereafter, dietary intakes should be sufficient to maintain maternal adiposity throughout gestation and thereby meet fetal nutrient requirements particularly during the final rapid growth phase. Gauging changes in skinfold thickness in addition to monitoring weight gain may be a simple and effective tool enabling health professionals to achieve this. Where adolescent pregnancies are unplanned and calorie intakes are predicted to be high, the mother should be advised of the dangers of excessive weight gain during pregnancy, particularly during the period spanning placental proliferation. Further, as the placenta is pivotal to mediating adverse pregnancy outcome in the young still-growing adolescent, early diagnosis of deficiencies in uteroplacental growth and/or blood flow by ultrasound are likely to be beneficial for identifying those at risk of fetal compromise and IUGR.

Using ART in the present study, we were able to largely uncouple pre-, peri- and post-conception nutritional exposures to judge their separate or interdependent influences. The clear conclusion from our novel design is that nutritional status at conception and nutritional intake thereafter influenced the placental growth trajectory, birth weight and the incidence of marked prenatal growth restriction, with nutritional intake after conception

having the most pronounced effect in sheep. Somewhat contrary to expectation, there was no evidence that pre-conception obesity (equivalent to ~33% body fat) negatively influenced embryo quality, conception rate or conceptus growth after embryo transfer into adolescent recipients. The public health implications specific to human adolescents of varying body fatness and growth status have been outlined previously. In addition, the data suggest that the focus for optimising the nutritional management of animals undergoing commercial ART for genetic improvement purposes should be the adiposity and gestational weight gain of the recipient rather than the adiposity status of the donor animal. Similarly those breeding ewes naturally in the first year of life (adolescents) need to appreciate that nutritional status at conception and dietary intake thereafter play an important and potentially modifiable role in maximising offspring birth weight and subsequent growth rate.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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