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Short Communication

Maternal insulin sensitivity in midpregnancy does not determine birth weight after embryo transfer between large and small breed sheep

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ABSTRACT

Embryo transfer of large sheep breed embryos (Suffolk) into small breed ewes (Cheviot) constrains birth size, but the maternal factors influencing fetal growth restriction are unknown. We hypothesized that reciprocal embryo transfer crosses between breeds of divergent size would affect pregnancy-related development of maternal insulin resistance in midgestation, thereby influencing fetal growth. Following superovulation, embryos were surgically collected 6 d postmating and transferred to recipients on the same day. Between- and within-breed transfers were performed. Between 60 and 70 d of pregnancy overnight-fasted ewes underwent hyperinsulinemic-euglycemic clamps for assessment of insulin sensitivity. Maternal insulin sensitivity did not vary with transferred lamb breed. Overall, Cheviot ewes tended to have higher fasting glucose ($P = 0.068$), fasting insulin ($P = 0.052$), and steady-state glucose ($P = 0.065$) concentrations than Suffolk ewes at the stage of pregnancy studied. As expected, transferred between-breed Suffolk lambs were born lighter ($P = 0.014$), and transferred between-breed Cheviot lambs tended to be heavier at birth ($P = 0.056$) than respective lambs transferred within breed. Midgestation insulin sensitivity does not appear to be a major factor constraining growth of large breed sheep fetus transferred into smaller breed or a factor in releasing constraint in growth of a small breed fetus within a larger breed ewe. However, as embryo size is already different between transferred groups by 19 d, factors other than maternal gestational insulin resistance may determine fetal growth in this embryo transfer paradigm.

1. Introduction

The role of uterine capacity in determining birth weight and size has been demonstrated in a number of cross-breeding experiments between large and small domestic animal species [1–4]. Embryo transfer experiments between large (Suffolk) and small (Cheviot) sheep breeds have offered a further refinement by excluding direct effects maternal genotype may have on outcome measures in the offspring [5–7]. These experiments are potentially very important in gaining a better understanding of the factors affecting size at birth, which in turn may have a profound effect on the health [8–11] and productivity of the offspring [12–14].

Although maternal breed size effects on birth size seem predictable from these embryo transfer experiments, the precise mechanisms regulating fetal growth in a constrained or a relatively unconstrained uterine environment

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remain elusive. Simple maternal uterine size is a possible factor, especially in late gestation, but follow-up studies on reciprocal embryo transfer between Cheviot and Suffolk sheep breeds have shown that fetal size is already affected by maternal size before 90 d gestation [6], well before spatial constraint of the maternal uterus could be expected to have full effect. It is highly likely that mechanisms to modulate fetal growth in a restricted or less restricted maternal environment are set early in pregnancy.

Earlier cohort studies of embryo transfer between Cheviot and Suffolk breeds suggest that maternal endocrine factors, such as placental lactogen (PL), progesterone, and insulin-like growth factor 1, are involved in the partitioning of nutrients and that key plasma metabolites, such as glucose and free fatty acids, are associated with the changes observed for the growth of transferred embryos [5]. Gestational hormones, such as PL [15] and progesterone [16], are thought to effect the development of gestational insulin resistance, a constitutive factor in mammalian pregnancy affecting the availability of glucose from the mother for placental transfer and, therefore, fetal growth [17]. In previous studies in sheep, we have demonstrated that maternal nutrition during the periconceptional period can alter the way gestational insulin resistance develops in midpregnancy [18] and, in turn, can affect fetal growth trajectory [19].

In this study, we tested whether altered maternal gestational insulin resistance was influenced in midgestation by transfer of Cheviot and Suffolk embryos between and within ewes of each breed shortly after conception. The aim of the study was to determine whether maternal gestational insulin resistance was an important factor modulating fetal growth in a mismatched maternal environment.

2. Materials and methods

2.1. Animal studies

Embryo transfer methods and their outcomes for the pure bred Cheviot and Suffolk animals used in this study have been reported in detail previously [5]. Briefly, 4-yr-old donor ewes provided embryos 6 d after artificial insemination while recipients were 4 to 6 yr; all ewes were multiparous. Standard commercial embryo transfer techniques were used to generate 4 treatments groups SinS (Suffolk embryo in Suffolk dam; large genotype control), CinC (Cheviot embryo in Cheviot dam; small genotype control), SinC (Suffolk embryo in Cheviot dam), and CinS (Cheviot embryo in Suffolk dam). From the previously mentioned transfers, we had 16 Cheviot ewes carrying 7 Cheviot and 9 Suffolk single embryos, and 16 Suffolk ewes carrying 9 Cheviot and 7 Suffolk single embryos with which to perform our studies.

All subsequent experiments were approved by the University of Auckland Animal Ethics Committee. Ewes were acclimatized to a full ration concentrate feed (3%–4 % of body weight per day, UniC, Dunstan, Hamilton, New Zealand) and indoor individual pens for 10 d before experiments were started. Body condition score within breed ranged between 3 and 4 at feedlot entry. In-house manufactured catheters were fitted to both jugular veins under local anesthesia (Xylocaine, Lignocaine 2%, AstraZeneca, Australia) and flushed with saline containing 10 U/mL heparin (Hameln Pharmaceuticals, Germany). Between 60 and 70 d of gestation (term = 145 d) ewes were fasted overnight before a hyperinsulinemic-euglycemic clamp was performed as described previously [18,20]. Following determination of baseline whole blood glucose concentrations (3 × 0.2 mL samples over 15 min) on a whole blood analyzer (YSI 2300, YSI Inc, Ohio, USA) a human insulin (Novo Nordisk, Denmark) infusion was started at a rate of 0.84 μM.kg/min. Blood samples (0.2 mL) were taken every 5 min. Glucose infusion commenced 15 min following insulin infusion and was titrated to return glucose concentrations to baseline. Blood samples (5 mL) were taken at times 0, 60, 75, 90, 105, and 120 min during the insulin infusion for measurement of insulin concentration. Infusions were stopped at 120 min. Insulin sensitivity of glucose (mM.nM.kg/min) was calculated by dividing the steady-state glucose infusion rate from 60 to 120 min by the steady-state concentration of plasma insulin over the same period [18].

At the completion of the clamps, the ewes were returned to pasture. Sex and birth weight of the lambs recorded was recorded within 1 d of birth.

2.2. Insulin assay

Ovine plasma insulin concentrations at baseline were measured by radioimmunoassay [21]. Human plasma insulin concentrations during the hyperinsulinemic-euglycemic clamp were measured by IMx insulin analyzer (Abbott diagnostics Division, Abbott Laboratories, Japan).

2.3. Statistical analysis

Only ewes in which steady-state concentrations of blood glucose during the hyperinsulinemic-euglycemic clamp were within 5% of baseline with a coefficient of variation 10% or less and plasma insulin concentration over the same period had a coefficient of variation 20% or less were included in the statistical analysis. The effects of maternal and fetal genotype on maternal outcomes were assessed using linear regression models, including lamb sex as a confounder. The interaction effect between maternal and fetal genotype was tested in all models. Models were also run including lamb birth weight as a covariate. Where necessary, parameters of glucose homeostasis were log-transformed to approximate normality. Analyses were performed in SAS v.9.3 (SAS Institute Inc, Cary, NC). Weight data in the table are presented as means ± standard deviations. All other data are presented as model-adjusted means with associated 95% confidence intervals.

3. Results

At the time of the clamp studies, Suffolk ewes were heavier than Cheviot ewes (74 kg [70–77 kg] vs 62 kg [58–66 kg]; $P < 0.001$). Maternal insulin sensitivity assessed by hyperinsulinemic-euglycemic clamp was not affected by transferred lamb breed at 60 to 70 d gestation