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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

**Table 1**
Characteristics of studied animals and the effects of embryo transfer on ewe glucose homeostasis.

| | Cheviot ewe | | Suffolk ewe | |
|---|---|---|---|---|
| | Cheviot lamb | Suffolk lamb | Cheviot lamb | Suffolk lamb |
| n | 7 | 9 | 9 | 7 |
| Ewe weight at clamp (kg) | 64.1 ± 5.1 | 59.7 ± 6.1 | 75.7 ± 8.0 | 71.3 ± 8.5 |
| Lamb birth weight (kg) | 5.18 ± 0.53 | 5.61 ± 1.31[a] | 5.68 ± 0.59 | 6.84 ± 0.97 |
| Lamb sex ratio (males:females) | 3:4 | 5:4 | 4:5 | 3:4 |
| Fasting glucose (mM) | 2.6 (2.4–2.9) | 2.6 (2.3–2.8) | 2.4 (2.2–2.6) | 2.4 (2.1–2.6) |
| Fasting insulin (nM) | 1.3 (0.5–2.8)[b] | 0.48 (0.24–0.96) | 0.31 (0.18–0.66) | 0.48 (0.24–1.08) |
| Steady-state glucose (mM) | 2.6 (2.3–2.8) | 2.5 (2.3–2.7) | 2.3 (2.1–2.5) | 2.3 (2.1–2.5) |
| Steady-state insulin (μM) | 1.2 (1.0–1.4) | 1.1 (0.9–1.3) | 1.3 (1.1–1.5) | 1.1 (0.9–1.4) |
| Steady-state glucose rate (mL/min) | 7.7 (7.1–8.2) | 7.2 (6.7–7.7) | 9.2 (8.4–9.9) | 8.6 (7.7–9.4) |
| Insulin sensitivity (mM.nM.kg/min) | 14 (11–18) | 18 (14–22) | 14 (10–20) | 14 (10–20) |

Weight data are means ± SD; all other data are model-adjusted means and 95% confidence intervals. “Steady-state glucose,” the mean plasma glucose concentration between 60 and 120 min of the clamp. “Steady-state insulin,” the mean plasma insulin concentration between 60 and 120 min of the clamp. “Stead-state glucose rate,” the mean glucose infusion rate between 60 and 120 min of the clamp. “Insulin sensitivity,” the steady-state glucose infusion rate divided by the steady-state concentration of plasma insulin.

[a] $P < 0.05$ and for a lamb breed effect within ewe breed.

[b] $P < 0.05$ for a ewe breed effect within lamb breed.

(Table 1). As would be expected, the smaller Cheviot ewes required a lower glucose infusion rate than Suffolk ewes (7.4 mL/min[7.0–7.9 mL/min] vs 8.9 mL/min [8.4–9.3 mL/min]; $P < 0.0001$). Overall, Cheviot ewes tended to have higher circulating fasting glucose (2.6 mM [2.5–2.8 mM] vs 2.4 mM [2.2–2.6 mM]; $P = 0.068$) and insulin (0.78 nM [0.48–1.38 nM] vs 0.36 nM [0.24–0.66 nM]; $P = 0.052$) concentrations, and higher steady-state glucose concentrations (2.5 mM [2.4–2.7 mM] vs 2.3 mM [2.2–2.5 mM]; $P = 0.065$) than Suffolk ewes.

A significant interaction between maternal and fetal genotypes was only observed for maternal fasting insulin ($P = 0.021$), with Cheviot ewes carrying Cheviot lambs having higher fasting insulin concentrations than Cheviot ewes carrying Suffolk lambs ($P = 0.019$; Table 1). Further, univariate analyses showed that lamb birth weight was inversely correlated with ewe fasting insulin ($r = -0.34$; $P = 0.027$), with a similar trend ($P = 0.072$) shown by the multivariate model.

Suffolk ewes gave birth to heavier lambs, irrespective of lamb breed (6.2 kg [5.8–6.7 kg] vs 5.4 kg [4.8–5.9 kg]; $P = 0.017$). Independently of ewe breed, Suffolk lambs were heavier than Cheviot lambs (6.2 kg [5.7–6.7 kg] vs 5.4 kg [4.9–5.9 kg]; $P = 0.024$), with a particularly marked difference seen among lambs born to Suffolk ewes ($P = 0.010$; Table 1). However, Suffolk lambs born of Cheviot ewes were lighter than Suffolk lambs born of Suffolk ewes ($P = 0.014$; Table 1). Similarly, Cheviot lambs born of Cheviot ewes tended to be lighter than Cheviot lambs born of Suffolk ewes ($P = 0.056$; Table 1).

## 4. Discussion

No difference in maternal insulin sensitivity at mid-pregnancy was found between large Suffolk breed ewes carrying transferred small Cheviot breed lambs or the reverse embryo transfer, or in comparison with within-breed transfers. This is despite constrained birth size of Suffolk lambs born to Cheviot ewes and a tendency for Cheviot lambs to be larger when born from the less constrained Suffolk uterine environment. Although fasting plasma insulin concentration was greater in CinC ewes, this did not manifest in a significant difference in integrated insulin sensitivity calculated from clamp data. We previously have reported that periconceptional events can influence the development of maternal insulin sensitivity at a similar stage of pregnancy [18] and trajectory of fetal growth later in pregnancy [19]. However, in the case of this large and/or small breed embryo transfer paradigm it appears that altered maternal insulin resistance in midgestation was not a critical factor driving the control of fetal growth. Many other factors are involved and perhaps events much earlier in pregnancy had more influence.

Correlative associations observed in previous studies using the Suffolk-Cheviot embryo transfer paradigm suggested pregnancy-related hormones PL and progesterone in the maternal circulation may play a role in influencing partitioning to favor fetal growth [5]. PL is thought to influence fetal and placental growth by increasing maternal insulin resistance [15] and also, perhaps, by stimulating lipolysis in the mother [22,23]. Maternal gestational insulin resistance is known to be an important factor affecting nutrient partitioning during pregnancy [17] and, in sheep, the maternal concentration of PL markedly increases as gestation progresses [24,25]. Progesterone, apart from its key roles in embryonic development and maintaining pregnancy, has also been suggested to have a role in the modulation of maternal insulin resistance during pregnancy [16]. Ewes which are undernourished from 60 d before to 30 d after mating have impaired development of maternal insulin resistance at 60 to 70 d of pregnancy [18] ewes have reduced plasma progesterone concentration at the same stage of gestation and lower PL concentrations detectable by 80 to 90 d of gestation [25]. However, in those same studies, despite the marked differences in maternal concentrations of progesterone and PL, and differing fetal growth trajectory, birth weight was not affected [19]. In the previous Cheviot-Suffolk embryo studies correlations of both hormones with birth size outcome were inconsistent; maternal values of PL at 90 d showed much less correlation with birth size compared with those measured at 120 d [5,26]. The similar maternal insulin sensitivity between

ewes carrying Cheviot or Suffolk fetuses at 60 to 70 d observed here may be consistent with the previously reported lack of difference in maternal plasma PL at a similar time in pregnancy.

There is the possibility that study of maternal insulin sensitivity later in gestation may have been more revealing given there is accelerated partitioning of maternal metabolites to the fetus. However, it is clear that differences in fetal size in the Cheviot-Suffolk embryo transfer paradigm in midgestation [6] are consistent with the birth data presented here and previously [5]. If maternal sensitivity during pregnancy was a key factor in determining fetal growth in this large and/or small breed embryo transfer one would expect evidence of this by midgestation. Any later appearance of altered maternal insulin sensitivity, if it were to occur, is unlikely to be the primary and original driver of altered birth size.

Other cohort studies using the same Cheviot-Suffolk embryo transfer paradigm have indicated that differences in transferred embryo length may occur as early as 19 d after conception [26]; however, these differences had disappeared by day 55 returning by day 90 [6]. This may suggest that events very early on set the trajectory for fetal growth to be accommodated in the available environment, rather than growth being clamped by simple spatial or metabolic constraint. This would be consistent with recent data demonstrating that size of birth in twin sheep is determined very early in pregnancy [27], presumably through conceptus-maternal signaling. Recent studies suggest that protein expression of both the progesterone and estrogen alpha receptor in the deep intercaruncular stroma and deep caruncular stroma of the Suffolk ovine uterus is reduced because of altered signaling from the Cheviot conceptus [7,28]. However, there were no changes reported in the same uterine tissues of the Cheviot ewe holding a Suffolk conceptus. Further studies need to focus on molecular signaling around the time of embryo transfer.

## 5. Conclusions

Within a model of reciprocal embryo transfer between small and large sheep breeds, no evidence was found to suggest a change in maternal insulin sensitivity in mid-pregnancy is the primary factor in determining birth size, or indeed fetal size effects of maternal environment observed in other cohorts, in mid-late gestation [6]. Although altered maternal insulin resistance does not appear to be the mechanism of maternal constraint in this embryo transfer paradigm, it remains a very important factor in the successful adaptation to pregnancy and the maintenance of fetal growth in all mammalian species studied to date.

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