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BRIEF COMMUNICATION: Does Viagra protect fetal lambs against maternal pregnancy toxemia?

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Introduction

Poor fetal growth is commonly caused by placental insufficiency. In sheep, multiple fetuses have relatively small placentae with fewer cotyledons than singletons, limiting blood flow to the placenta, especially in late gestation when fetal growth rate is highest. Factors that reduce fetal growth, such as maternal genotype, nutrition, age, parity, fetal number, or environmental temperature, typically reduce placental size and are associated with lower rates of fetal oxygen and nutrient uptakes and placental blood flow (Reynolds & Redmer 2001). Further, circumstances that influence placental vascular development have a dramatic impact on fetal growth and development, and affect neonatal survival and growth (Borowicz et al. 2007; Satterfield et al. 2010).

Sildenafil citrate (SC, trade names Viagra or Avigra) increases vasodilatation of uteroplacental vessels (Wareing et al. 2005) and may represent a novel therapy to improve fetal lamb growth. Sildenafil citrate has successfully increased birth weight in rodent models and singleton-bearing ewes (Satterfield et al. 2010; Miller et al. 2009). There is no published information regarding the use of SC in multiple-bearing ewes. The objective of this trial was to determine if SC administration to triplet-bearing ewes in late gestation could increase lamb birth weight. In addition, the influence of SC on the incidence of pregnancy toxemia is also reported here.

Materials and methods

This experiment was conducted at AgResearch Palmerston North, with approval of the AgResearch Animal Ethics Committee.

Following a five-day acclimatization period when ewes were transitioned from a pasture-only diet to a pelleted diet, 36 triplet-bearing ewes were moved indoors at day 100 of pregnancy (P100), allocated at random to one of six pens and fed a commercial lucerne-based sheep pellet (Uni C) containing 9.5 MJME/DM in amounts greater than the calculated requirements of each pen (based upon weekly weights). At P110, six ewes that had gained the least weight were removed from the trial. From P110 to P140, ewes in pens 1-3 (n=15) were injected with 50 mg SC whilst those in pens 4-6 (control C; n=15) received vehicle (water) three times daily (at 8-h intervals). Treatments

were administered via indwelling subcutaneous catheters in a 9 ml volume. From P126 all pens were supplemented three times daily with 1 kg (3 kg/pen/d) Fiber Ezy (Fiber Fresh Feeds Ltd, Reporoa, New Zealand), containing (according to label) 13 MJME/kg. Refusals were collected and recorded daily. Ewe blood samples were collected at P117, P128 and P137 and serum/plasma stored at -20°C for assay of glucose (hexokinase method) β -hydroxybutyrate (enzyme/colourimetric), and NEFA (enzymatic Acyl-CoA synthetase substrate). From P135, eight SC and two C ewes exhibited sub-clinical signs of pregnancy toxemia (confirmed by plasma β -hydroxybutyrate concentrations) and at P145 fresh cut grass was supplied to all ewes *ad libitum* and feed measurement ceased. One ewe (in SC group) underwent caesarean section and was removed from the trial. Within eight hours of birth, lambs were weighed, tagged, and crown-rump, girth, limb lengths measured, and placentas collected and dissected.

Statistical analysis

Statistical analysis was completed using REG (Gilmour, 1990). Mixed models included fixed effects of treatment and variable effects of ewe weight, lamb sex, date of birth and ME intake. The Chi-squared test was used to test the effect of treatment on lamb survival.

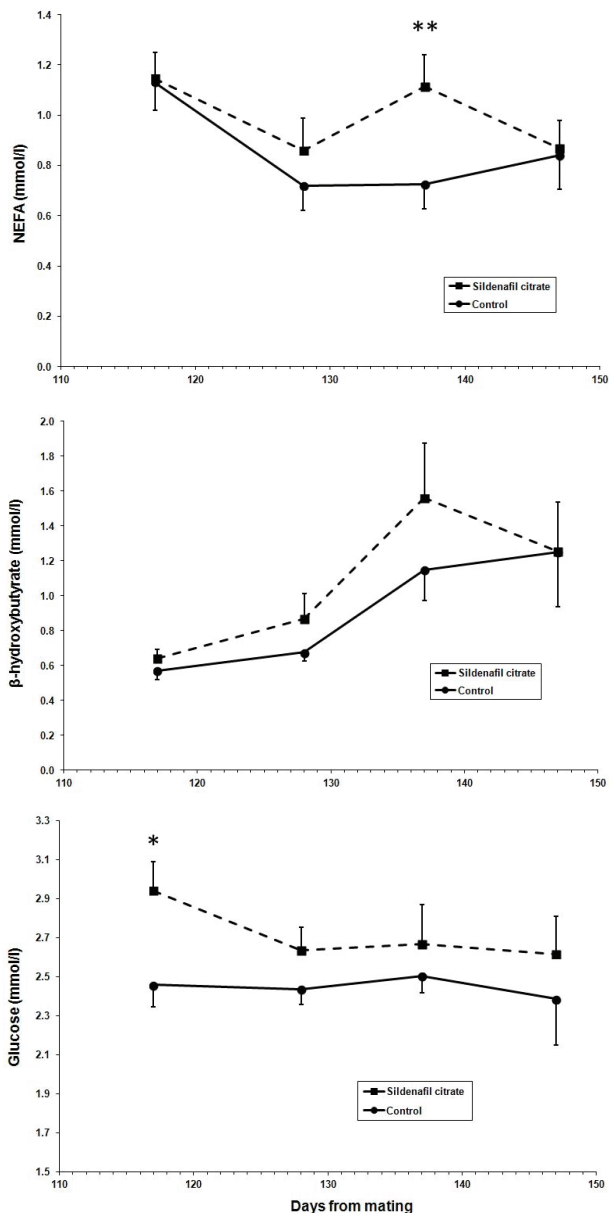
Results

Thirty-seven lambs were born alive in the SC group (14 ewes) and 22 in the C group (15 ewes) (Table 1). Twelve ewes in the SC group each gave birth to three live lambs whilst only five ewes in the C group delivered three live lambs (X^2 6.7; $P=0.01$). Amongst only ewes whose full set of triplet lambs were all born alive, treatment had no effect on total weight of lambs born per ewe after adjustment for date of parturition (which was significant). However, when data was analysed for all lambs (n=87) SC lambs were significantly heavier ($P<0.01$) than C lambs. There was no treatment effect on placental weight, membrane and cotyledon weight or cotyledon number. SC ewes consumed less ($P=0.01$) energy (795 \pm 52 MJME) than C ewes (889 \pm 65 MJME). Plasma glucose concentrations were higher ($P<0.05$) in the SC group at P117 (a week after treatments started) but were not different ($P>0.05$) thereafter (Figure 1). Plasma β -hydroxybutyrate concentrations did not differ during the treatment period, but serum NEFA concentrations

Table 1 Effect of subcutaneous injection of triplet-bearing ewes with 50 mg sildenafil citrate or water (vehicle) three times daily from P110-P140 on survival of lambs, birth weights of lambs, weights of placentas and placental components. Values are means±sem.

	Sildenafil citrate	n	Water (control)	n	P
Number of ewes	14		15		
Number of ewes with all three lambs born alive	13		5		P<0.01
Number of lambs born	42		45		
Number of lambs born alive	37 (88%)		22 (49%)		P<0.01
Date of parturition	23 Sept	14	19 Sept	15	P<0.01
Weight of ewe (kg)	74.7±1.5	14	73.1±1.5	15	NS
Weight of lambs per ewe (kg)	11.26±0.59	14	10.00±0.48	15	NS
Individual lamb birth weight (kg)	3.75±0.13	42	3.34±0.11	45	0.013
Weight of placenta (g)	747±83	11	794±82	13	NS
Weight of fetal membranes (g)	476±53	11	515±52	13	NS
Weight of cotyledons (g)	219±30	11	248±29	13	NS
Number of cotyledons	86±9	11	84±7	13	NS

Figure 1 Serum NEFA, plasma β-hydroxybutyrate, and glucose concentrations in triplet-bearing ewes treated with 50 mg sildenafil citrate or water (vehicle) three times daily for thirty days (P110- P140) during late gestation. * P<0.05 **P<0.01



were significantly higher (P<0.01) in SC ewes than in C ewes at P136, ten days before parturition (Figure 1).

Discussion

Sildenafil citrate-treated ewes ate less but delivered heavier lambs, with a later date of birth and a greater proportion of live births, without apparent effects on placental weight or anatomy. SC has apparently protected the ewes and fetuses from metabolic stress and thus, prolonged gestation and allowed more lambs to survive. Higher plasma glucose concentrations after one week and higher serum NEFA ten days before parturition indicate that SC has effected a change in metabolism of the ewes. One hypothesis is that SC has altered nutrient partitioning sufficient to ameliorate pregnancy toxemia. Furthermore, small changes in blood concentrations of nutrients may result in large increases in nutrient uptake by the uteroplacental unit due to increases in blood flow. Other metabolites (not measured) may have been involved, e.g., polyamines, critical mediators of placental growth, were found in higher concentration in fluids of SC-treated fetuses than in controls by Satterfield et al. (2007). Another possibility is that amino acid availability was changed; Satterfield et al. (2007) found greater concentrations of total amino acids in amniotic fluid, allantoic fluid, and fetal umbilical venous serum, as well as a higher ratio of total amino acids in serum from fetal umbilical vein to uterine artery. Parenteral arginine administration has been shown to stimulate fetal growth presumably by increasing blood flow (Lassala et al. 2010; McCoard et al. 2016). Alternatively, SC may have direct metabolic actions on metabolism in ewes and fetuses; SC treatment for three months increased insulin sensitivity in humans (Ramirez et al. 2015). Further study is required to confirm effects and elucidate causal relationships in pregnant ewes.

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