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BRIEF COMMUNICATION: Do lambs within a twin and triplet-born litter produce different amounts of heat during a cold stress event?

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INTRODUCTION

Triplet-born lambs have been shown to have lighter birth weights (Morris & Kenyon, 2004; Kerslake *et al.*, 2005; Everett-Hincks & Dodds, 2008), lower rectal temperatures (Dwyer & Morgan, 2006; Stafford et al., 2007), lower plasma fructose (Stafford et al., 2007) and greater plasma lactate concentrations within five minutes of birth (Stafford et al., 2007; J.I. Kerslake, Unpublished data) than twin-born lambs. In addition to this, within a triplet-born litter, the lightest lambs have been shown to have lower rectal temperatures and greater plasma lactate concentrations within five minutes of birth (Stafford et al., 2007; J.I. Kerslake, Unpublished data) than the heaviest lambs. Light birth weights, low rectal temperatures and greater plasma lactate concentrations within five minutes of birth have been used as indices for placental insufficiency, acute intra-partum hypoxemia and inadequate thermogenesis after birth (Barlow et al., 1987; Mellor, 1988). These physiological characteristics can result in poor thermoregulation during the post-natal period and may offer some explanation as to why triplet-born lambs have a greater mortality rate than twin-born lambs and why the lightest lambs within a triplet-born litter have a greater mortality rate than their heavier siblings. Previous research which has compared the heat production capacity of different litter sizes have shown mixed results, with maximum heat production on per kg of body weight basis being the same between single-, twin- and a small number of triplet-born lambs (Alexander, 1962), and maximum heat production on a per kg of body weight basis being greater in single-born lambs compared to twin-born lambs (Stott & Slee, 1987). The heat production capacity of lambs within twin- or triplet-born litters has not been examined. Therefore the aim of this investigation was to compare the heat production capacity of twin- and triplet-born lambs, and to determine if lambs within twin- or triplet-born litters produce different amounts of heat during a cold stress event.

MATERIALS AND METHODS

Thirty five days prior to mating half of a mob of 1,150 mixed-age Romney cross ewes (n = 575) were randomly selected and each ewe injected intramuscularly in the anterior half of the neck with 1.5 mL of iodised peanut oil (Flexidine® (26% w/w of iodine bound to ethyl esters of unsaturated fatty acids in oil), Bomac Laboratories Ltd, Auckland, New Zealand) (Supplemented). The other half of the mob was not injected (Non-supplemented). From mating until day 68 of pregnancy (P68), the ewes were grazed on *ad-libitum* ryegrass *(Lolium perenne)* and white clover *(Trifolium repens)* pasture. At P68 30 twin- and 30 triplet-bearing ewes were randomly selected from both the Supplemented and Non-supplemented mobs. These groups were then further split with 14 twin- and 16 triplet-bearing ewes from the Supplemented and Non-supplemented groups being grazed on kale from P68 until P120, and then on *ad-libitum* ryegrass and white clover pasture until parturition. The remaining 16 twin- and 14 triplet-bearing ewes were grazed on *ad-libitum* ryegrass and white clover pasture from P68 until parturition.

On P145, all ewes that had been walked around twice daily and habituated to the presence of humans for one week before lambing were moved into a 2.5 ha paddock and supervised continuously for ten days. Ewes that showed signs of parturition were moved quietly to a 5 m by 3 m temporary pen located at the front of their paddock. The ewes remained in these pens during parturition with the ewes and lambs released from the pen 24 hours after the last lamb was born. All ewes had access to herbage and water within the pen. Ewes which gave birth in the paddock remained there, with no observations recorded and no measurements taken.

At 24 to 36 hours after birth, 18 twin (Supplemented, n = 2; Non-supplemented, n = 16) and 34 triplet (Supplemented, n = 17; Non-supplemented, n = 17) lambs born to ewes grazing kale followed by pasture, and 28 twin (Supplemented, n = 14; Non-supplemented, n = 14) and 30 triplet (Supplemented, n = 17; Non-supplemented, n = 13) lambs born to ewes grazing pasture only were randomly selected to determine their maximum heat production or summit metabolism, by indirect open-circuit calorimetry. Prior to the calorimentric measurements being taken a 5 mL blood sample was collected by jugular venepuncture (lithium heparin and potassium oxalate vacutainer, Becton Dickson Vacutainer Systems, USA) for the measurement of gamma glutamyl transferase (GGT) as an indicator of colostrums intake. During indirect open-circuit calorimetry lambs were exposed to wet and windy conditions to induce maximum heat production. Artificial chilled rain (1°C) was applied through sprinklers at a standardized rate of (1.0 L/min) and cold air passed over the lamb by a fan positioned behind the animal at a speed of 1.0 m/sec. After 20 minutes the speed of the cold air was increased to 1.5 m/sec and after another 20 minutes increased further to 2.0 m/sec. The speed of the cold air then stayed constant for the remaining period of time. Rectal temperature and oxygen consumption measurements were taken at 4 minute intervals for 88 minutes or until the lamb reached maximum heat production, whichever occurred first. Maximum heat production was assumed to have been met when the rectal temperature of lamb declined at the rate of 1°C/20 minutes and there was no further increase in the consumption rate of oxygen (Alexander, 1962). After the lamb reached maximum heat production or after 88 minutes in the calorimeter, the lamb was removed and dried. Lambs were then placed in a heated room for 1 hour before being returned to their dams. Metabolic rate in watts (W) and watts/kg live weight (W/kg LW) were measured as previously described by Kerslake *et al.* (2009).

For statistical analyses lambs from twin- and triplet-born litters were categorised from lightest to heaviest with respect to their relative weight within the litter as; heavy-triplet, medium-triplet, light-triplet, heavy-twin and light-twin. This is an approach previously used by Stafford *et al.* (2007), Kenyon *et al.* (2007), Morel *et al.* (2008) and Morel *et al.* (2009). A general linear model (PROC GLM, SAS, 2003) was used to identify any significant differences between maternal treatments, and between and within birth ranks for lamb physical measurements of birth weight, surface-area-to-body-weight ratio, crown-rump length and thoracic-girth circumference, and lamb heat production measurements of maximum heat production on a per lamb basis and a per kg of live weight basis. These models contained the fixed effects of maternal iodine supplementation (Supplemented, Non-supplemented), ewe nutrition (Kale, Pasture), lamb birth rank (Twin, Triplet), and their interactions. The fixed effect of birth rank (Twin, Triplet), along with its interactions, were nested within birth weight category (Heavy, Medium, Light). Lamb birth weight was fitted as a covariate. This identified whether the dependent variables were influenced more by lamb birth rank, lamb birth weight or relative size within litter. The interactions or

TABLE 1: The effect of birth rank (Twin, Triplet) and relative size within litter (Heavy-twin, Light-twin, Heavy-triplet, Medium-triplet, Light-triplet) on lamb birth weight and surface-area. Data is presented as mean ± standard error.

Parameter	Number of lambs	Birth weight (kg)	Surface area (m^2)
Birth rank			
Twin	96	4.8 ± 0.06	0.298 ± 0.004
Triplet	138	3.9 ± 0.06	0.269 ± 0.003
P value		0.001	0.001
Relative size within litter			
Twin			
Heavy	48	5.1 ± 0.08^d	0.311 ± 0.005^a
Light	48	4.5 ± 0.09^c	0.285 ± 0.005^b
Triplet			
Heavy	46	4.4 ± 0.10^c	0.292 ± 0.005^b
Medium	46	4.0 ± 0.10^b	0.270 ± 0.004^c
Light	46	3.4 ± 0.11^a	0.246 ± 0.005^d
P value		0.001	0.001

[1]Surface area = 0.121 x Birth weight)$^{0.59}$ (Pierce, 1934). Differing superscripts within columns of relative size within litter indicate significant differences (P <0.05).

TABLE 2: The effect of birth rank (Twin, Triplet) and relative size within litter (Twin-Heavy, Twin-Light, Triplet-Heavy, Triplet-Medium, Triplet-Light) on lamb maximum heat production on a per lamb basis and on per kg of birth weight basis. Data is presented as means ± standard errors.

Parameter	Number of lambs	Maximum heat production	
		Per lamb (W/lamb)	Per unit of birth weight (W/kg)
Birth rank			
Twin	35	71.0 ± 3.8	15.2 ± 0.7
Triplet	56	61.2 ± 2.9	15.4 ± 0.6
P value		0.050	0.840
Twin			
Heavy	18	76.7 ± 5.2^b	15.3 ± 1.0
Light	17	65.2 ± 5.3^b	15.2 ± 1.0
Triplet			
Heavy	22	72.6 ± 4.6^b	16.0 ± 0.9
Medium	20	64.2 ± 4.8^b	16.1 ± 0.9
Light	14	46.8 ± 5.8^a	14.1 ± 1.1
P value		0.003	0.485

Differing superscripts within columns of relative size within litter indicate significant differences (P <0.05).

covariates only remained in the general linear model if significant ($P < 0.05$). To adjust for differences in environmental temperatures within the first 24 hours, the average cold stress index within the first 24 hours of the lambs' life was calculated and fitted as a covariate in all lamb heat production models. Plasma GGT concentrations at 24 to 36 hours of age was also fitted as a covariate in an attempt to adjust for differences in colostrum intake.

Statistical analyses showed that maternal iodine supplementation, ewe nutrition and their potential interactions had no effect ($P > 0.05$) on lamb physical measurements and lamb heat production measurements (J.I. Kerslake, Unpublished data). This communication only discusses the differences between birth ranks and within twin- and triplet litters.

RESULTS AND DISCUSSION

The lightest triplet born lamb had a birth weight which was approximately 15 to 23% lower than the heaviest and medium triplet-born lambs, but only a 8 to 14% decrease in surface area. Similarly it had a birth weight which was approximately 25 to 34% lower than twin-born lambs, but only a 14 to 20% decrease in surface area (Table 1). This suggests that the lightest triplet-born lambs would lose the greatest amount of heat to the environment during a cold stress event (Alexander, 1962). Triplet-born lambs also produced less heat on a per lamb basis than twin-born lambs, and the lightest-triplet-born lamb produced less heat on a per lamb basis than the heaviest-twin, lightest-twin, heaviest-triplet-, and medium-triplet-born lamb (Table 2). Once heat production on a per lamb basis was adjusted for lamb birth weight however, all twin- and triplet-born lambs within a litter produced the same amount of heat (pooled means ± standard deviation were 63.8 ± 21.7 W and 15.2 ± 3.9 W kg^{-1}), where a one kg increase in lamb birth weight was associated with a 12.4 (± 3.81(Standard error)) W increase in maximum heat production. This indicates that within a litter, the birth rank or relative size of the lamb within the litter had no affect on heat production at 24 to 36 hours of age. Instead, it is the weight of the lamb that drives heat production.

These findings are in disagreement with Stott and Slee (1987), who suggested that greater metabolic rates were a characteristic of litter size and not simply a reflexion of lamb birth weight. These findings are however, in agreement with those of Alexander (1962) and Kerslake *et al.* (2009), who showed that the maximum heat production on a per kg of body weight basis was the same in single-, twin- and triplet-born lambs. In the current experiment, the heat production capacity of the lamb on a per kg of birth weight basis (W/kg) was 8% less than the rates measured by Eales and Small (1980), and 18 to 24% less than the rates measured in the experiments of Alexander (1962), Stott and Slee (1987) and Kerslake *et al.* (2009). While depressed heat production between birth and 12 hours after birth, can result from prolonged hypoxemia during pregnancy or acute hypoxemia during the birth process, as observed by Eales and Small (1980), depressed heat production at 24 to 36 hours after birth is more likely to result from limited energy reserves, failure of the ewe to provide sufficient milk and/or failure of the lamb to suck (Stott & Slee, 1985). The ewes and lambs in the current experiment were penned from birth to 24 hours after birth and had blood samples taken at birth and three and 24 hours after birth, and rectal temperature measurements taken at birth, and one, three, 12 and 24 hours after birth as part of another trial. This disruption may have disturbed the mother-lamb bond and sucking time of the lamb. In a similar experiment, where ewes and lambs were not penned for the first 24 hours after birth, heat production capacity on a per kg of body weight were 18% higher than the current experiment (Kerslake *et al.* 2009). Differences between experiments may have also resulted from different methodologies to determine maximum heat production and/or the breed of the lamb as Merino vs. Scottish Blackface vs. Romney.

Overall the results suggest that the lightest-triplet-born lamb would lose the greatest amount of heat during a cold stress event, and that they would also produce less heat than all twin-born lambs and the heaviest- and medium-triplet-born lambs. This is a reflection of lamb birth weight, and not its birth rank or relative size within its litter. Ensuring greater lamb birth weights within twin- and triplet-born lamb litters should improve the thermoregulatory capabilities of the newborn lambs and potentially improve their survival rates.

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