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BRIEF COMMUNICATION: Is placentome size more important than morphologic type in relation to fetal weight in sheep?

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Keywords: caruncle; cotyledon; placentome size; placentome type; singleton; twin

Introduction

In sheep, placental exchange of nutrients between the ewe and her fetus occurs at sites called placentomes (Ford 2000). Placentomes are comprised of cotyledons on the chorioallantoic membrane which attach to discrete caruncles on the uterine wall (Ford 2000). Ovine placentomes can be classified into four types on the basis of their gross morphological appearance (Vatnick et al. 1991). The distribution of placentome type and size of individual placentomes can be influenced by many factors such as, nutritional state, placental blood flow and oxygen availability (Gardner et al. 2002; Osgerby et al. 2004). In general, previous work has shown that adverse intrauterine conditions early in pregnancy lead to a shift to more everted placentomes (more C and D types) (Osgerby et al. 2004; Vonnahme et al. 2006). This has led to the suggestion that the presence of more everted placentomes is an adaptation to increased nutrient delivery to a compromised fetus (Hoet & Hanson 1999). However, more recent work from Vonnahme et al. (2008) has shown that placentome size is more important than morphological type with respect to vascularity, suggesting that placentome size may be more important for nutrient transfer than type. To date, it is still unclear what is most important in relation to fetal weight in sheep; morphologic type or size of the placentome.

It is well known that a twin uterine environment differs from a singleton environment. At term foetuses within a twin pair may reflect differences in the degree of growth retardation they have experienced (Clarke et al. 2000). We, and others, have reported placental data for twins in sheep on a twin-pair basis, assuming that no differences within twin-pair in placental weight exist, even when the twins within a pair differ in weight (McCoard et al. 1997; Vonnahme et al. 2006; Vonnahme et al. 2008). To date, little information is available on the placentome type and size distribution per twin fetus within a twin-pair.

The objective of this study was to investigate the relationship between placentome morphologic type and size with fetal weight in late pregnancy within individual placentae of singleton and twin fetuses and between fetuses within a twin-pair.

Materials and methods

The study and all animal handling procedures were approved by the AgResearch Animal Ethics Committee, Palmerston North, New Zealand in accordance with the 1999 Animal Welfare Act of New Zealand.

Ten singleton-bearing and six twin-bearing ewes, aged 3- and 4-years-old at approximately 90 days of pregnancy (P90), were selected from a commercial Romney flock at AgResearch Ballantrae farm. Selected ewes were offered *ad libitum* grazing as one group on pasture with a minimum of 1,100 kg DM/ha under New Zealand commercial grazing conditions. One singleton-bearing ewe lambed prior to P140 and was excluded from the study.

At P140, ewes were euthanized by captive bolt immediately followed by exsanguination. After the fetus was removed from the uterus, placentomes per individual placenta were dissected from the uterus and classified into four types (Type A placentomes are concave in shape with the maternal tissue completely surrounding the fetal tissue; Type B placentomes consist of fetal tissue beginning to grow over the surrounding maternal tissue; Type C placentomes are flat and consist of a large portion of fetal tissue that has begun to surround the maternal tissue; Type D placentomes contain mostly fetal tissue, which surrounds the maternal tissue), based on the morphological classification system described by Vatnick et al. (1991). If the types were "intermediate" they were considered to be the more advanced type. The placentomes were manually separated into their caruncular and cotyledonary components and each component weighed individually.

Based on cotyledonary weight, individual placentomes were classed into Light (0 to 2.0 g), Medium (2.1 to 4.0 g) or Heavy (> 4.1 g) groups. The placentae of one twin-pair could not be separated by fetus, as both fetuses were located in the same horn. Data from these placentae were therefore omitted from analysis for placental characteristics.

To determine within-twin effects on placental characteristics, fetuses with the lightest body weight within a pair were classified as Light ($n = 5$) and the heaviest were classified as Heavy ($n = 5$).

Table 1 Mean \pm standard error of fetal weight, fetal:placentome weight ratio and placental parameters of placentomes of singleton and twin fetuses and Light and Heavy fetuses in a twin pair at Day 140 of pregnancy. Bold type indicates significance at $P < 0.05$. Italic type indicates significance between $P = 0.05$ and $P = 0.09$.

Measurement	Between pregnancy rank			Within twin pair		
	Singleton (n = 9)	Twin (n = 10)	P value	Light (n = 5)	Heavy (n = 5)	P value
Fetal weight (g)	4,779 \pm 140	4,022 \pm 173	0.006	3,889 \pm 174	4,154 \pm 174	0.05
Fetal:placentome weight ratio	13.8 \pm 1.1	16.2 \pm 1.3	0.19	14.8 \pm 1.7	17.7 \pm 1.7	0.07
Total number of placentomes	88.4 \pm 5.6	57.2 \pm 5.4	0.001	62.2 \pm 5.3	52.2 \pm 5.3	0.22
Total placentome weight (g)	360.7 \pm 25.5	258.7 \pm 32.7	0.03	271.7 \pm 24.8	245.7 \pm 24.8	0.22
Total caruncular weight (g)	77.8 \pm 3.9	52.0 \pm 4.7	0.002	55.2 \pm 4.7	48.8 \pm 4.7	0.19
Total cotyledonary weight (g)	282.8 \pm 24.3	206.7 \pm 31.7	0.08	216.5 \pm 22.1	196.9 \pm 22.1	0.24

Statistical analysis

Fetal body weight and within-twin pair fetal body weight were analysed using the MIXED procedure (SAS 2006) with a linear model that included the fixed effects of either pregnancy rank or fetal size (Heavy or Light) and the random effect of ewe. Placental parameters were analysed using the MIXED procedure with a linear model that included the fixed effects pregnancy rank or fetal size, placentome type (A to D), placentome size (Light, Medium or Heavy) and the random effects of ewe and fetus. Data were presented as least square means \pm standard error. Only one placenta contained Type D placentomes. Type D placentomes were therefore omitted from the analysis.

Results and discussion

Placental characteristics between singletons and twins

Placentae of individual twin-fetuses had fewer placentomes, less total placentome weight and total caruncular weight and tended ($P = 0.083$) to have less total cotyledonary weight compared to the placentae

of singletons (Table 1). The fetal:placentome weight ratio was numerically ($P = 0.190$) greater for twin fetuses compared to singleton fetuses. No differences between singleton and twin placentae were found for total placentome, caruncular or cotyledonary weight or number within placentome type (DS van der Linden, Unpublished data). Placentae of twin fetuses had a greater proportion of Type B placentomes than placentae of singletons (Fig. 1). Singleton placentae had a greater proportion of Type A and B than C placentomes, whereas, twin placentae were mainly occupied by Type B placentomes. Placentae of singletons tended ($P = 0.07$) to have a greater proportions of Light placentomes compared to placentae of twins (Fig. 2). Twin placentae were occupied by a greater proportion of Medium placentomes than Light placentomes. No differences in placentome size were found within singleton placentae. These results indicate that the functionality of the placentome types and sizes may be different as proportions of placentome size and type were differently distributed between placentae of singletons and twins. These findings agree with the suggestion that more everted placentomes develop in

Figure 1 Distribution of placentome type (%) for placentae of singletons (n = 9) and twins (n = 10) and Light (L-twin; n = 5) and Heavy (H-twin; n = 5) fetuses within a twin pair at Day 140 of gestation. Data are expressed as least square mean \pm standard error of proportion of each placentome type group.

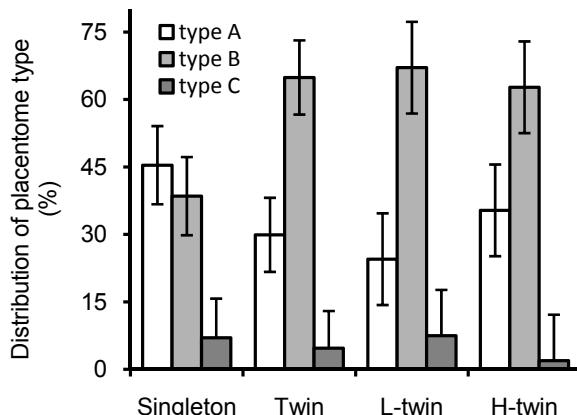
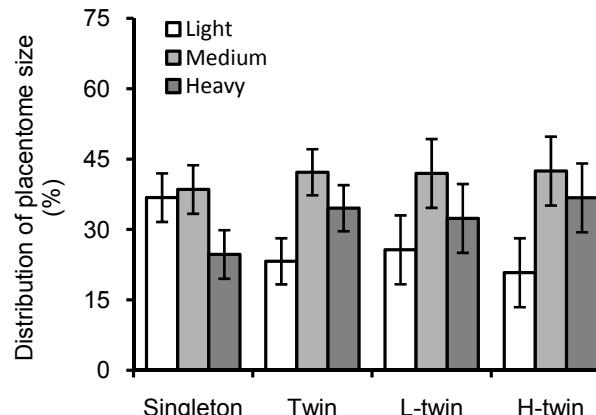


Figure 2 Distribution of placentome size (%) for placentae of singletons (n = 9) and twins (n = 10) and Light (L-twin; n = 5) and Heavy (H-twin; n = 5) fetuses within a twin pair at Day 140 of gestation. Data are expressed as least square mean \pm standard error of proportion of each placentome size group.



sub-optimal uterine conditions in an attempt to increase nutrient transport to the fetus (Hoet & Hanson 1999). Although it is believed that placental nutrient delivery is increased in more everted placentomes, there is still relatively little information on placental nutrient delivery with respect to placentome type and, in particular placentome size. Therefore, when investigating placental development and fetal growth, future research should emphasize on placentome size in conjunction morphologic type.

Placental characteristics within a twin-pair

Fetal weight within a twin pair tended ($P = 0.053$) to be different, however, no differences were observed for total placentome weight or number per placentome type within twins (Table 1). No differences between placentae of Light and Heavy fetuses were found for total placentome, caruncular or cotyledonary weight and number, irrespective of placentome type (DS van der Linden, Unpublished data). No differences in the proportion of placentome type or size were found between placentae of Light and Heavy fetuses (Fig. 1). The placentae of Light fetuses had a greater proportion of Type B placentomes compared to Type A and C. Heavy fetuses had a greater proportion of Type A and B compared to C placentomes. In addition, Heavy fetuses had a greater proportion of Medium and Heavy placentomes compared to Light placentomes, but no such differences were observed in the placentae of Light fetuses. Nevertheless, the Heavy twin tended ($P = 0.067$) to have a more efficient placenta, as reflected in a greater fetal:placentome weight ratio, compared to the Light twin; thus more fetal weight per unit of placental weight. This contrasts the fetal:placentome weight ratio of the (heavier) singletons with the (lighter) twins. Eberle et al. (1993) showed in human twins that the lighter twin is as likely to have the larger part of the placenta as the smaller part. This indicates that reduced nutrient transfer by a smaller placenta does not seem to be solely responsible for a decrease in twin weight (Eberle et al. 1993). These results may indicate that the two placentae of twins are not the same as two individual singleton placentae.

In conclusion, given that fetal weight may be more dependent on the distribution of placentome size and type it might be necessary for future studies to focus on placentome size in conjunction with morphologic type, when investigating placental functionality and fetal growth in sheep. In addition, more research is needed to investigate nutrient transport across placentomes to be able to make conclusive statements about placentome type and size. Within a twin pair, the placentae showed altered placental efficiency, indicating that two placentae of twins are not the same as two individual singleton placentae. Therefore, the common practice of considering one placenta of a twin pair as a single unit may lead to false conclusions.

Acknowledgements

The authors are grateful to AgResearch Capability Postdoctoral Fellowship Fund for funding the project. The authors would also like to thank John Napier, Trevor Holloway and Hailey Gillespie for their help with the animal work and Maria Loureiro and Amy Paten for their help with tissue collection. We also gratefully acknowledge the National Research Centre for Growth and Development for providing a scientific writing retreat where this manuscript has been prepared.

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