Fetal programming by co-twin rivalry in sheep[1]

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ABSTRACT: Fetal rivalry for space and nutrients compromises intrauterine environment and fetal growth, this leading to further consequences during adult life (i.e., fetal programming). Focusing on sheep, relevant fetal programming effects have been revealed on body composition and growth although little is known about their potential impact on the reproductive performance of adult ewes. This research focused on the analysis of fetal programming-related effects on 41,475 litter size (LS) records from 7,177 purebred Ripollesa ewes. Fetal programming sources of variation accounted for the linear and quadratic effect of absolute birth BW (ABBW), relative birth BW (RBBW) of twin-born ewes (i.e., both magnitude and direction of the birth BW difference between the ewe and its co-twin), and sex of twin ewe's littermate (SLM). More specifically, data were analyzed under a threshold mixed model and the statistical relevance of models accounting for different combinations of ABBW, RBBW, and SLM effects was compared by Bayes factors (BF; i.e., the ratio between the posterior probability of 2 competing models). The model accounting for RBBW and discarding both ABBW and SLM effects was clearly preferred; its posterior probability was 35.2 to 362.3 times higher than from remaining models and provided very strong ($31.6 < \text{BF} < 100$) and decisive evidences ($\text{BF} > 100$) supporting the relevance of RBBW and the negligibility of both ABBW and SLM. Single-born ewes were included as reference group and they reached a predicted LS of 1.189 lambs per lambing. Twin-born ewes being >600 g lighter than their co-twins suffered from an impaired reproductive ability with 1.162 lambs per lambing (95% credible interval [95CI], 1.147 to 1.179), and this estimate increased until ewes were 151 to 300 g lighter than their co-twins (1.226 lambs per lambing; 95CI, 1.208 to 1.244). Remaining categories (i.e., ewes being heavier or equal than their co-twins) did not provide significant differences and showed an enhanced reproductive ability of approximately 1.23 lambs per lambing. These significant differences in LS highlighted the influence of fetal programming in sheep under rangeland conditions, which implies decisive economic consequences worldwide. Moreover, these results could contribute additional information on twin biology, which could be useful in other mammalian species such as humans.

Key words: birth weight, fetal programming, litter size, Ripollesa sheep

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J. Anim. Sci. 2014.92:64–71
doi:10.2527/jas2013-6677

INTRODUCTION

The "fetal programming" theory suggests that intrauterine environment may alter expression of the fetal genome, this resulting in developmental adaptations that permanently modify the activity of the hypothalamic-pituitary-gonadotropic axis as well as the development of several tissues and organs (Barker and Clark, 1997; Wu et al., 2006). These effects have been previously revealed in sheep, in which they modified the pituitary and ovary function (Da Silva et al., 2001; Wallace et al., 2001; Fowden et al., 2005) and body composition and growth (Greenwood et al., 1998a, 1998b). Nevertheless, little is known about their impact on the reproductive performance of adult ewes, with the exception of some evidences regarding fetal development of gonads (Da Silva et al., 2002, 2003) and uterus (Sosa et al., 2009), puberty onset (Da Silva

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[1] The authors are indebted to the Associació Nacional de Criadors d'Oví de Raça Ripollesa (Monells, Spain) for providing field data. Funding for this study was provided by a research contract with the Departament d'Agricultura, Ramaderia, Pesca, Alimentació i Medi Natural of the Catalonia's government (Barcelona, Spain). The research contract of J. Casellas was partially financed by Spain's Ministerio de Economía y Competitividad (Madrid, Spain) within the context of the Ramón y Cajal program (RYC-2009-04049).

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Received May 7, 2013.
Accepted October 29, 2013.

et al., 2001), and fertility (Long et al., 2010). Litter size (**LS**) is one of the most important traits for the meat sheep industry (Olesen et al., 1994) and the presence of fetal programming effects, if any, could be of economic relevance for stockbreeders worldwide.

Despite most of the studies on fetal programming having been conducted under artificially induced aggressions (e.g., maternal undernutrition; Kotsampasi et al., 2009; Long et al., 2010), in utero environment is also affected by physiological processes such as twinning. Fetal programming could acquire a decisive relevance within this context, where a variable degree of prenatal environmental competition is widely known due to zygosity, cotiledonary-placental size and caruncule number (Alexander, 1964), cojoined placental circulation and sex interactions between fetuses (Valdés Cruz et al., 1977; Wilkes et al., 1978), and transuterine embryonic migration of embryos and physical location in utero (Donald and Purser, 1956; Scanlon, 1972). The objective of this research was to evaluate the incidence of twinning-related fetal programming long-term effects on LS in the Ripollesa sheep breed.

MATERIALS AND METHODS

Animal Care and Use Committee approval was not obtained for this study because analyses were performed on existing field data obtained under standard farm management from commercial breeders. Both productive and reproductive data were collected by the stockbreeders themselves and administrated by the Ripollesa breed society [Associació Nacional de Criadors d'Oví de Raça Ripollesa (**ANCRI**), Monells, Spain] within the context of its performance recording scheme.

Field Data Source

The analysis of fetal programming effects in sheep focused on LS data from Ripollesa ewes. The Ripollesa breed is a representative example of meat-type sheep managed under semi-extensive Mediterranean conditions in northeast Spain, the "pascual"-type lamb being its typical commercial product (22 to 24 kg BW at slaughter). This is a medium-sized sheep breed (rams, 75 to 90 kg BW; ewes, 50 to 65 kg BW) with dark brown or black marks on the head and legs and white semi-fine wool with closed fleece (Esquivelzeta et al., 2011).

Analyses were performed on 41,475 LS records (single, 81.09%; twins, 18.41%; triplets, 0.47%; quadruplets, 0.02%) from 7,177 purebred Ripollesa ewes collected during 38 yr (1975 to 2012) in 11 flocks with pedigree connection. All flocks were active members of ANCRI and contributed data to the official performance recording scheme. All Ripollesa ewes taking part in the recording scheme were individually identified by ear tags and, after 1995, intraruminal electronic identification boluses; they were administrated per os at the age of joining the flock as replacement breeding stock (Caja et al., 1999; Ghirardi et al., 2006). All relevant reproductive and productive data (e.g., ewe identification number, lambing date, number of lambs born, lamb identification number, birth BW, and sex of the lamb) were registered by the shepherds themselves. For analytical purposes, LS was redefined as a dichotomous variable with response categories of single (i.e., 1 lamb per lambing) and multiple lambings (i.e., >1 lamb per lambing) in order to avoid extreme categories with too few data.

Analytical Model

Three different potential sources of fetal programming-related effects on LS data were investigated in this study, the first 2 being determined by ewe's BW at birth (**BBW**). First of all, linear and quadratic effects of absolute BBW (**ABBW**) were considered as plausible indicators of fetal programming effects linked to overall in utero damages (e.g., placental malfunctions; Wallace et al., 2005) and benefits (e.g., maternal nutrition; Gardner, 2007). The impact of fetal rivalry on the experienced in utero environment was assessed by accounting for the relative BBW (**RBBW**). Both the magnitude (i.e., <150 g, 151 to 300 g, 301 to 450 g, 451 to 600 g, and >600 g) and direction (i.e., the ewe being the lighter or heavier co-twin) of the BBW difference between the ewe and its co-twin was characterized into 9 categories as shown in Table 1; an extra category for ewes from single births was also considered. Although the incidence of freemartin individuals in the ovine species is almost null (Padula, 2005), the last fetal programming-related effect classified twin birth-ewes into a dichotomous effect depending on the sex of their littermate (**SLM**; male or female). It is important to note that freemartin ewes are typically reported as infertile (Power et al., 1985; Gill and Davies, 1991); in this study, infertile ewes that were not capable of getting pregnant were not considered and the influence of SLM, if any, was evaluated in terms of realized LS from fertile ewes.

The 3 previous systematic effects were evaluated into a standard threshold mixed model (Wright, 1934; Sorensen et al., 1995) where unobservable liabilities inherent to LS records (**u**; see below for details on threshold model parameterization under a Bayesian framework) were modeled as

$$\mathbf{u} = \mathbf{X}_1\mathbf{b}_1 + \mathbf{X}_2\mathbf{b}_2 + \mathbf{Z}_1\mathbf{p}_1 + \mathbf{Z}_2\mathbf{p}_2 + \mathbf{e}.$$

Note that $\mathbf{b}_1$ was the vector of fetal programming-related effects, $\mathbf{b}_2$ was the vector accounting for the re-

Table 1. Summary of the Ripollesa data set on the basis of the potential intrauterine competition. Ewes were grouped depending on their type of birth (i.e., single or twin) and twin-born ewes subdivided into 9 additional categories regarding the birth weight difference with their littermate and the direction of the difference (i.e., ewe being the heavier or the lighter co-twin)

Categories of experienced intrauterine competition			Ewes			Lambings	
Type of birth	Heavier or lighter co-twin (if applicable)	Birth BW difference between co-twins, g (if applicable)	*n*	Averagebirth BW, kg	Male siblings, % (if applicable)	*n*	Litter size, lambs per lambing
Single	–[1]	–	4,566	$4.12^{a} \pm 0.01$	–	27,231	$1.160^{e} \pm 0.002$
Twin	Lighter	>600	289	$3.09^{g} \pm 0.04$	$64.7^{a} \pm 2.8$	1,607	$1.199^{d} \pm 0.010$
Twin	Lighter	451 to 600	189	$3.24^{f,g} \pm 0.05$	$56.9^{a,b} \pm 3.6$	1,174	$1.201^{d} \pm 0.012$
Twin	Lighter	301 to 450	115	$3.33^{e,f} \pm 0.06$	$64.0^{a,b} \pm 4.5$	550	$1.284^{a,b} \pm 0.019$
Twin	Lighter	151 to 300	344	$3.43^{d,e} \pm 0.03$	$52.8^{b,c} \pm 2.7$	1,860	$1.251^{b,c} \pm 0.010$
Twin	–	<150	830	$3.50^{d} \pm 0.02$	$41.9^{d} \pm 1.7$	4,561	$1.288^{a} \pm 0.007$
Twin	Heavier	151 to 300	207	$3.66^{c} \pm 0.04$	$34.0^{d} \pm 3.3$	1,026	$1.218^{c,d} \pm 0.013$
Twin	Heavier	301 to 450	218	$3.71^{c} \pm 0.04$	$42.4^{c,d} \pm 3.4$	1,075	$1.254^{a,b} \pm 0.013$
Twin	Heavier	451 to 600	108	$3.63^{c} \pm 0.05$	$42.1^{c,d} \pm 4.8$	630	$1.198^{d} \pm 0.016$
Twin	Heavier	>600	311	$3.92^{b} \pm 0.04$	$41.6^{d} \pm 2.8$	1,761	$1.208^{d} \pm 0.010$
Overall	–	–	7,177	3.90 ± 0.01	47.3 ± 0.6[2]	41,475	1.189 ± 0.002

[a–f] Within-column estimates with the same superscript do not differ significantly ($P > 0.05$). Two-tailed *t*-tests (average birth BW and litter size) and *Z*-tests (percentage of male siblings) were used.

[1] Not applicable.

[2] Only twin-born ewes were considered.

maining systematic effects, $\mathbf{p}_1$ and $\mathbf{p}_2$ were vectors accounting for 2 independent permanent environmental effects (see below for additional details), $\mathbf{X}_1$, $\mathbf{X}_2$, $\mathbf{Z}_1$, and $\mathbf{Z}_2$ were appropriate incidence matrices, and $\mathbf{e}$ was the vector of residual terms. More specifically, $\mathbf{b}_1$ accounted for a specific combination of fetal programming-related effects (i.e., ABBW, RBBW, and SLM; 8 possible combinations are described below), $\mathbf{b}_2$ accounted for the age of the ewe at lambing (<18 mo, 18 to 29 mo, 30 to 41 mo, 42 to 53 mo, 54 to 65 mo, and >65 mo) and lambing interval (ewe-lambs, <210 d, 210 to 239 d, 240 to 329 d, and >329 d), $\mathbf{p}_1$ accounted for flock-year-season effects with 320 levels, and $\mathbf{p}_2$ accounted for the permanent environmental effect inherent to each ewe (7,177 levels). Genetic effects were not accounted for because pedigree data was scarce, with 94% of unknown paternities.

Bayesian Inference

Although we evaluated different combinations for ABBW, RBBW, and SLM effects in order to elucidate their biological and statistical relevance (see below), the following Bayesian development describes the most complex model accounting for all of them. We assumed that LS was linked to an unobservable continuous variable ($\mathbf{u}$; i.e., liability) that followed a multivariate normal distribution (Sorensen et al., 1995; Van Tassell et al., 1998),

$$p(\mathbf{u}|\mathbf{b},\mathbf{p}) = \mathrm{MVN}(\mathbf{X}_1\mathbf{b}_1 + \mathbf{X}_2\mathbf{b}_2 + \mathbf{Z}_1\mathbf{p}_1 + \mathbf{Z}_2\mathbf{p}_2, \mathbf{I}),$$

in which $\mathbf{I}$ was an identity matrix with dimensions equal to the number of LS records and that followed a multivariate normal (**MVN**) distribution. Note that LS data were analyzed on a dichotomous scale and the residual variance was therefore arbitrarily fitted to 1 (Sorensen et al., 1995). Within this context, the contribution to the likelihood of the *i*th LS record (y_i) conditional on the *i*th element in vector $\mathbf{u}$ (u_i) was assumed as

$$p(y_i|u_i) = I(y_i = 1) \times I(u_i < 0) + I(y_i = 2) \times I(u_i \geq 0),$$

in which $I(\,)$ was an indicator function with the argument defined in parentheses. The indicator function had value of 1 if the evaluated expression was true and value of 0 if not. A priori distributions for permanent environmental effects were assumed multivariate normal,

$$p(\mathbf{p}_1|\sigma_1^2) = \mathrm{MVN}(\mathbf{0}|\mathbf{I}_1\sigma_1^2) \quad \text{and}$$

$$p(\mathbf{p}_2|\sigma_2^2) = \mathrm{MVN}(\mathbf{0}|\mathbf{I}_2\sigma_2^2),$$

in which σ_1^2 and σ_2^2 were appropriate variance components, $\mathbf{I}_1$ (and $\mathbf{I}_2$) was an incidence matrix with dimensions equal to the number of elements in vector $\mathbf{p}_1$ (and $\mathbf{p}_2$), and $\mathbf{0}$ were zeroing vectors. On the other hand, flat priors with boundaries at –1,000 and 1,000 were assumed for all systematic effects.

Inferences for all the unknown parameters in the model were made on their relevant marginal posterior distribution by Gibbs sampling (Gelfand and Smith, 1990). For each model, a Monte Carlo Markov chain

(**MCMC**) with 1,010,000 iterations was launched and the first 10,000 were discarded as burn-in. Indeed, convergence of each model was evaluated on variance components (i.e., σ_1^2 and σ_2^2) by visual inspection of the chains and by applying the method of Raftery and Lewis (1992). In all cases, convergence was reached before iteration 1,000 although a larger burn-in period was assumed to guarantee stationary sampling. A total of 20,000 samples of model parameters were saved with a lag interval of 50 iterations; these samples were used to characterize the posterior distribution of each model parameter invoking the ergodic property of the chain (Gilks et al., 1996). Given that inference was made on unobservable liabilities instead of observable LS records (Sorensen et al., 1995), model estimates from systematic effects were transformed to the observable scale by applying the method described by Hansen et al. (2004). More specifically, the predicted LS (LS_{ij}) inherent to the jth level of the ith systematic effect ($b_{1,ij}$) can be obtained by applying

$$\mathrm{LS}_{ij} = 1 + \Phi(\Phi^{-1}(0.189) + b_{1,ij}),$$

in which 0.189 was the overall incidence of multiple lambings in the Ripollesa data set (Table 1) and $\Phi(\,)$ was the cumulative distribution function of a standard normal distribution with the argument as described within parenthesis. Note that this expression assumed that all multiple lambings were twins.

Testing for Fetal Programming-Related Effects

The Bayes factor (**BF**) is the basic tool for comparing 2 competing models in the Bayesian framework (Kass and Raftery, 1995). This statistic provides the ratio between the posterior probabilities inherent to each model and highlights the advantage of the numerator ($\mathrm{BF} > 1$) or the denominator model ($\mathrm{BF} < 1$). Within this context, 8 different models were defined by assuming the following structures of $\mathbf{b}_1$:

$\mathbf{b}_1$: 0,

$\mathbf{b}_1$: ABBW,

$\mathbf{b}_1$: RBBW,

$\mathbf{b}_1$: SLM,

$\mathbf{b}_1$: ABBW + RBBW,

$\mathbf{b}_1$: ABBW + SLM,

$\mathbf{b}_1$: RBBW + SLM, and

$\mathbf{b}_1$: ABBW + RBBW + SLM.

The BF was computed for each of the 28 pairwise comparisons between models with different $\mathbf{b}_1$ parameterizations by applying the approximation described by Newton and Raftery (1994). The statistical relevance of each BF was classified by assuming Jeffreys' (1984) levels of evidence, that is, $\mathrm{BF} < 1$ (denominator model supported), $1 < \mathrm{BF} < 3.16$ (barely worth mentioning), $3.16 < \mathrm{BF} < 10$ (substantial evidence), $10 < \mathrm{BF} < 31.6$ (strong evidence), $31.6 < \mathrm{BF} < 100$ (very strong evidence), and $\mathrm{BF} > 100$ (decisive evidence).

Focusing on the model with highest BF estimates, the posterior distribution of each fetal programming-related effect was summarized in terms of posterior mean and 95% Bayesian confidence interval (i.e., credible intervals; **95CI**). More specifically, the posterior mean of each model parameter was calculated by averaging MCMC samples and after discarding the burn-in period whereas 2.5th and 97.5th percentiles characterized the 95CI.

RESULTS AND DISCUSSION

Field Data on Potential Fetal Programming Factors

Before going deep into long-term effects on LS, fetal programming-related factors and their interrelationships should be outlined from field data. Twin-born ewes included in this study averaged 3.51 ± 0.02 kg BW at birth, this value matching with the average BBW of twin-born female lambs in the Ripollesa breed (3.47 ± 0.01 kg BW; $n = 14{,}706$) and discarding genetic selection for heavier ewe-lambs at birth in this population. Moreover, male co-twins were slightly heavier (3.57 ± 0.01 kg BW; $n = 15{,}134$) as previously reported in Rambouillet, Targhee, and Columbia breeds (Burfening, 1972) as well as in the Soay sheep (Korsten et al., 2009); note that opposite differences have also been reported in wild ruminants such as the Saiga antelope (*Saiga tatarica* L.; Kühl et al., 2007). As shown in Table 1, twin-born ewes were lighter than single-born ewes (4.12 ± 0.01 kg BW; $P < 0.05$) and the average BBW evolved across categories of relative BBW, lighter and heavier estimates agreeing with smallest and highest relative BBW, respectively. Indeed, both absolute and relative BBW were positively correlated ($r = 0.380$; $P < 0.05$) as suggested in Fig. 1. This positive relationship must be viewed as an anticipatable consequence of the biological limit for litter weight inherent to all polytocous breeds.

Almost half of the twin-born ewes had a male co-twin (47.3%) although this percentage significantly increased up to 64.7% for ewes being the smallest co-twin at birth with a relative BBW difference larger than 600 g (Table 1). This suggested that largest BBW differences were linked to different-sex twins, female co-twins being smaller as reported by Burfening (1972) and Korsten et al. (2009) in other sheep breeds. This illustrates a complex scenario where all potential fetal-programming factors showed substantial interrelationships among them, and detailed analyses become essential to elucidate the contribution of each factor as well as to prevent biases and unrealistic estimates.

Model Comparison

Making comparisons between models is a topic of major interest in animal science, given the substantial impact that a model can have on estimated effects and subsequent conclusions. Within this context, BF were calculated to decide whether fetal programming-related effects had statistically relevant impact on the reproductive performance of adult ewes, both as separate effects or integrated into the same model. The null model without fetal contributions was clearly discarded and obtained lower-than-1 BF in all pairwise comparison when placed as denominator (Table 2). Although without going into detail, this evidenced that LS performance of adult ewes was influenced to some degree by the experienced fetal environment, a long time ago. This agreed with previous evidences reviewed in multiple domestic species and productive traits (Wu et al., 2006; Funston et al., 2010), where fetal programming effects were revealed as a very relevant source of variation.

The contribution of ABBW, RBBW, and SLM to LS was tested by comparing models with all possible combinations of fetal programming-related effects. As shown in Table 2, the sole inclusion of RBBW was clearly preferred and provided very strong (BF > 31.6) and decisive evidences (BF > 100) against remaining models. It is important to highlight that even the inclusion of other fetal programming-related effects along with RBBW did not improve model performance and discarded relevant contributions from ABBW or SLM. Indeed, these effects reached barely irrelevant BF estimates when implemented alone (BF < 2) and their 95CI included the null estimate in all cases (results not shown); although some marginal influences on LS might have been captured by them as suggested by the larger-than-1 BF, they should not be considered as the real source of fetal programming influences in later life. Note that these results disagree with Korsten et al. (2009), who linked both fetal programming-related effects to the reproductive fitness of Soay ewes although RBBW was not considered in that study. In the Ripollesa sheep, the statistical preeminence of RBBW advocated for biological mechanisms linked to co-twin rivalry in utero, whatever the sex of the lambs and the absolute BW they reach at birth. Note that RBBW could be viewed as a relevant example

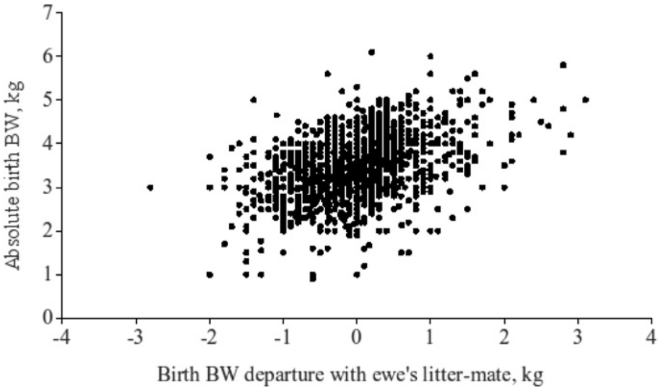

Figure 1. Plot of absolute birth BW against birth BW departure with the littermate for twin-born ewes.

Table 2. Bayes factors comparing threshold models for litter size data in the Ripollesa breed; differences between models relied on the inclusion or exclusion of fetal programming-related effects such as absolute (ABBW) and relative birth BW (RBBW) and sex of the co-twin littermate (SLM)

Denominator model	Numerator model[1]							
	0	ABBW	RBBW	SLM	ABBW + RBBW	ABBW + SLM	RBBW + SLM	ABBW + RBBW + SLM
0	1	2.9	362.3	1.6	4.7	2.0	4.1	10.3
ABBW	0.3	1	125.1	0.6	1.6	0.7	1.4	3.6
RBBW	<0.1	<0.1	1	<0.1	<0.1	<0.1	<0.1	<0.1
SLM	0.6	1.8	222.0	1	2.9	1.2	2.5	6.3
ABBW + RBBW	0.2	0.6	76.9	0.3	1	0.4	0.9	2.2
ABBW + SLM	0.5	1.4	177.7	0.8	2.3	1	2.0	5.0
RBBW + SLM	0.2	0.7	87.5	0.4	1.1	0.5	1	2.5
ABBW + RBBW + SLM	0.1	0.3	35.2	0.2	0.5	0.2	0.4	1

[1] The base model (0) accounted for ewe age at lambing, lambing interval, flock-year-season, and ewe's permanent environment whereas remaining models expanded this parameterization by including fetal programming-related effects.

of intrauterine growth retardation, a phenomenon previously linked to fetal programming effects on a plethora of biological functions of the adult life in livestock (Wu et al., 2006). Moreover, this also suggests that intrauterine growth retardation and its consequences must be modeled on the basis of within-gestation BW deviations instead of the BW itself in twins (and may be polytocous) gestations.

Fetal Programming-Related Effects on Litter Size

From now on, estimates from the analysis of LS will refer to the parameterization with highest BF (see Table 2), this being the model accounting for RBBW and discarding ABBW and SLM effects. It is important to note that RBBW included single-born ewes as reference and evaluated the effect of fetal rivalry on twin-born ewes. Within this context, predicted LS from single-born ewes reached an intermediate value (1.189 lambs per lambing) and most of the categories involving twin-born ewes evidenced statistically relevant departures around this estimate (Table 3). More specifically, those twin-born ewes being >600 g lighter than their co-twins suffered from an impaired reproductive ability with 1.162 lambs per lambing (95CI: 1.147 to 1.179) whereas twin-born ewes being 300 g or less lighter than their co-twins enhanced reproductive ability up to estimates around 1.23 lambs per lambing (Table 3). This pattern evidenced that reproductive ability was remarkably impaired for animals with lowest relative birth BW although this biological function improved quickly until reaching a plateau remarkably higher than single-born ewes' performance. These results characterized the complex relationship between BBW and fetal programming, showing that reductions in BBW were only informative when comparing with siblings; that is, twin ewes were not doomed to reproductive failure per se, even assuming a raw starting penalty in their birth weight.

Our results showed the dramatic effect of fetal programming on lighter co-twins, which could be related to an early impaired ovarian and follicular development (Rae et al., 2001). Note that previous results in Rhesus macaques (*Macaca mulatta* L.) suggested similar fetal programming consequences in ovaries although these effects were not induced by physiological processes such as twin pregnancies but artificial hyperandrogenism (Abbott et al., 2006, 2008). It is difficult to elucidate the physiological pathways underlying reproductive departures in our Ripollesa ewes although current state-of-the-art about fetal programming postulates permanent alterations in hormonal axes as causal mechanisms. The ability of the early environment to program the hypothalamo-pituitary-adrenal axis has been documented in several species (Matthews, 2002). Moreover, there is a remarkable interplay between the hypothalamo-pituitary-adrenal and the hypothalamo-pituitary-gonadal axes, and emerging evidence indicates that this interaction is also modified by early environmental manipulation (Abbott et al., 2008; Kotsampasi et al., 2009). These influences could be mediated by variations in fetal thyroxin and corticosteroid profiles (Kotsampasi et al., 2009). Both of these hormones are known to affect brain development in the fetus, inducing permanent changes in function (Matthews, 2002; Abbott et al., 2008; Kotsampasi et al., 2009). It is important to highlight that previous research on reproductive fetal programming in sheep has focused on experimentally undernourished ewes (Rae et al., 2001) whereas this study reports substantial effects of fetal programming under standard feeding and management conditions, which could imply relevant economic consequences in the ovine sector worldwide.

Table 3. Posterior mean and 95% credible interval (95CI) for the percentage of multiple lambing across categories of intrauterine competition. Estimates were transformed to the observable scale by applying the method described by Hansen et al. (2004)

Categories of experienced intrauterine competition			Predicted LS,[1] %	
Type of birth	Heavier or lighter co-twin (if applicable)	Birth BW departure between co-twins, g (if applicable)	Posterior mean	95CI
Single	–[2]	–	1.189^{b}	
Twin	Lighter	>600	1.162^{a}	1.147 to 1.179
Twin	Lighter	451 to 600	1.175^{a,b}	1.156 to 1.194
Twin	Lighter	301 to 450	1.215^{b,c}	1.187 to 1.246
Twin	Lighter	151 to 300	1.226^{c}	1.208 to 1.244
Twin	–	<150	1.237^{c}	1.224 to 1.250
Twin	Heavier	151 to 300	1.233^{c}	1.210 to 1.256
Twin	Heavier	301 to 450	1.235^{c}	1.213 to 1.258
Twin	Heavier	451 to 600	1.231^{c}	1.203 to 1.261
Twin	Heavier	>600	1.229^{c}	1.195 to 1.249

$^{a–c}$Estimates with different superscript do not overlap their 95CI.

[1] LS = litter size.

[2] Not applicable.

On the other hand, we must be cautious with the advantage of (most of) twin-born ewes when comparing with single-born ewes. It is well known that placental efficiency increases with LS (Dwyer et al., 2005), and this phenomenon could be responsible of the higher incidence of multiple lambing in twin-born ewes being 300 g or less lighter than their co-twin. Nevertheless, we cannot discard other effects such as an anticipatable genetic superiority of twin-born ewes (Casellas et al., 2007); although these genetic effects and other environmental sources of variation could be accounted for by the permanent environmental effect inherent to each ewe, they cannot be completely discarded.

Although these results cannot be directly extrapolated to humans, sheep could be viewed as a reasonable model to analyze fetal programming in co-twins. Significant influences of fetal programming on human health and child survival have been reported (Barker and Clark, 1997), and our results also suggest a relevant penalty on the reproductive function under physiological conditions. Fetal programming due to intrauterine competition for resources is a fact that must be dealt with in sheep, with important implications for the selection of replacement stock. This study could be a starting point for future studies on twin rivalry in other species. Understanding these mechanisms could hold the key to the development of therapeutic interventions aimed at reversing the impact of an adverse intrauterine or neonatal environment on the reproductive function.

LITERATURE CITED

Abbott, D. H., D. K. Barnett, J. E. Levine, V. Padmanabhan, D. A. Dumesic, S. Jacoris, and A. F. Tarantal. 2008. Endocrine antecedents of polycystic ovary syndrome in fetal and infant prenatally androgenized female rhesus monkeys. Biol. Reprod. 79:154–163.

Abbott, D. H., V. Padmanabhan, and D. A. Dumesic. 2006. Contributions of androgen and estrogen to fetal programming of ovarian dysfunction. Reprod. Biol. Endocrinol. 4:17.

Alexander, G. 1964. Studies on the placenta of the sheep (*Ovis aries* L.). Effect of surgical reduction in the number of caruncles. J. Reprod. Fertil. 7:307–322.

Barker, D. J. P., and P. M. Clark. 1997. Fetal undernutrition and disease in later life. Rev. Reprod. 2:105–112.

Burfening, P. J. 1972. Prenatal and postnatal competition among twin lambs. Anim. Prod. 15:61–66.

Caja, G., C. Conill, R. Nehring, and O. Ribó. 1999. Development of a ceramic bolus for the permanent electronic identification of sheep, goat and cattle. Comput. Electron. Agric. 24:45–63.

Casellas, J., G. Caja, A. Ferret, and J. Piedrafita. 2007. Analysis of litter size and days to lambing in the Ripollesa ewe. II. Estimation of variance components and response to phenotypic selection on litter size. J. Anim. Sci. 85:625–631.

Da Silva, P., R. P. Aitken, S. M. Rhind, P. A. Racey, and J. M. Wallace. 2001. Influence of placentally-mediated foetal growth restriction on the onset of puberty in male and female lambs. Reproduction 122:375–383.

Da Silva, P., R. P. Aitken, S. M. Rhind, P. A. Racey, and J. M. Wallace. 2002. Impact of maternal nutrition during pregnancy on pituitary gonadotrophin gene expression and ovarian development in growth-restricted and normally grown late gestation sheep fetuses. Reproduction 123:769–777.

Da Silva, P., R. P. Aitken, S. M. Rhind, P. A. Racey, and J. M. Wallace. 2003. Effect of maternal overnutrition during pregnancy on pituitary gonadotrophin gene expression and gonadal morphology in female and male sheep at day 103 of gestation. Placenta 24:248–257.

Donald, H. P., and A. F. Purser. 1956. Competition in utero between twin lambs. J. Agric. Sci. 48:245–249.

Dwyer, C. M., S. K. Calvert, M. Farish, J. Donbavand, and H. E. Pickup. 2005. Breed, litter and parity effects on placental weight and placentome number, and consequences for the neonatal behaviour of the lamb. Theriogenology 63:1092–1110.

Esquivelzeta, C., M. Fina, R. Bach, C. Madruga, G. Caja, J. Casellas, and J. Piedrafita. 2011. Morphological analysis and subpopulation characterization of Ripollesa sheep breed. Anim. Genet. Resour. 49:9–17.

Fowden, A. L., D. A. Giussani, and A. J. Forhead. 2005. Endocrine and metabolic programming during intrauterine development. Early Hum. Dev. 81:723–734.

Funston, R. N., D. M. Larson, and K. A. Vonnahme. 2010. Effects of maternal nutrition on conceptus growth and offspring performance: Implications for beef cattle production. J. Anim. Sci. 88:E205–E215.

Gardner, D. S. 2007. Factors affecting birth weight in sheep: Maternal environment. Reproduction 133:297–307.

Gelfand, A., and A. F. M. Smith. 1990. Sampling based approaches to calculating marginal densities. J. Am. Stat. Assoc. 85:398–409.

Ghirardi, J. J., G. Caja, D. Garín, M. Hernández-Jover, O. Ribó, and J. Casellas. 2006. Retention of different sizes of electronic identification boluses in the forestomachs of sheep. J. Anim. Sci. 84:2865–2872.

Gilks, W. R., S. Richardson, and D. J. Spiegelhalter. 1996. Markov chain Monte Carlo in practice. Chapman and Hall, London, UK.

Gill, J. J. B., and D. A. R. Davies. 1991. XX/XY chromosomal chimerism in infertile sheep of the Cambridge breed. Genet. Sel. Evol. 23:S126–S130.

Greenwood, P. L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 1998a. Effects of birth weight and postnatal nutrition on neonatal sheep: I. Body growth and composition, and some aspects of energetic efficiency. J. Anim. Sci. 76:2354–2367.

Greenwood, P. L., A. S. Hunt, J. W. Hermanson, and A. W. Bell. 1998b. Effects of birth weight and postnatal nutrition on neonatal sheep: II. Skeletal muscle growth and development. J. Anim. Sci. 78:50–61.

Hansen, M., I. Misztal, M. S. Lund, J. Pedersen, and L. G. Christensen. 2004. Undesired phenotypic and genetic trend for stillbirth in Danish Holsteins. J. Dairy Sci. 87:1477–1486.

Jeffreys, H. 1984. Theory of probability. 3rd ed. Clarendon Press, Oxford, UK.

Kass, R. E., and A. E. Raftery. 1995. Bayes factors. J. Am. Stat. Assoc. 90:773–795.

Korsten, P., T. Clutton-Brock, J. G. Pilkington, J. M. Pemberton, and L. E. B. Kruuk. 2009. Sexual conflict in twins: Male co-twins reduce fitness of female Soay sheep. Biol. Lett. 5:663–666.

Kotsampasi, B., S. Chadio, G. Papadomichelakis, S. Deligeorgis, D. Kalogiannis, I. Menegatos, and G. Zervas. 2009. Effects of maternal undernutrition on the hypothalamic-pituitary-gonadal axis function in female sheep offspring. Reprod. Domest. Anim. 44:677–684.

Kühl, A., A. Mysterud, G. I. Erdnenov, A. A. Lushchekina, I. A. Grachev, A. B. Bekenov, and E. J. Milner-Gulland. 2007. The ‘big spenders’ of the steppe: Sex-specific maternal allocation and twinning in the Saiga antelope. Proc. Biol. Sci. 274:1293–1299.

Long, N. M., M. J. Nijland, P. W. Nathanielsz, and S. P. Ford. 2010. The effect of early to mid-gestational nutrient restriction on female offspring fertility and hypothalamic-pituitary-adrenal axis response to stress. J. Anim. Sci. 88:2029–2037.

Matthews, S. G. 2002. Early programming of the hypothalamo-pituitary-adrenal axis. Trends Endocrinol. Metab. 13:373–380.

Newton, M. A., and A. E. Raftery. 1994. Approximate Bayesian inference by the weighted likelihood bootstrap (with discussion). J. R. Stat. Soc., B 56:1–48.

Olesen, I., M. Pérez-Enciso, D. Gianola, and D. L. Thomas. 1994. A comparison of normal and nonnormal mixed models for number of lambs born in Norwegian sheep. J. Anim. Sci. 72:1166–1173.

Padula, A. M. 2005. The freemartin syndrome: An update. Anim. Reprod. Sci. 87:93–109.

Power, M. M., S. Hanrahan, and O. O’Reilly. 1985. Cytogenetic assessment of chimerism in infertile sheep. J. Dairy Sci. 68(Suppl. 1):250 (Abstr.).

Rae, M. T., S. Palassio, C. E. Kyle, A. N. Brooks, R. G. Lea, D. W. Miller, and S. M. Rhind. 2001. Effect of maternal undernutrition during pregnancy on early ovarian development and subsequent follicular development in sheep fetuses. Reproduction 122:915–922.

Raftery, A. E., and S. M. Lewis. 1992. How many iterations in the Gibbs sampler? In: J. M. Bernardo, J. O. Berger, A. P. Dawid, and A. F. M. Smith, editors, Bayesian statistics IV. Oxford Univ. Press, Oxford, UK. p. 763–773.

Scanlon, P. F. 1972. Frequency of transuterine migration of embryos in ewes and cows. J. Anim. Sci. 34:791–794.

Sorensen, D. A., S. Andersen, D. Gianola, and I. Korsgaard. 1995. Bayesian inference on threshold models using Gibbs sampling. Genet. Sel. Evol. 27:229–249.

Sosa, C., J. A. Abecia, M. Carriquiry, F. Dorcada, G. B. Martin, I. Palacin, and A. Meikle. 2009. Early pregnancy alters the matabolic responses to restricted nutrition in sheep. Domest. Anim. Endocrinol. 36:13–23.

Valdés Cruz, L. M., M. J. Taylor, J. C. Mott, and J. G. Craver. 1977. Detection of conjoined placental circulation in twin fetal lambs. J. Reprod. Fertil. 51:503–505.

Van Tassell, C. P., L. D. Van Vleck, and K. E. Gregory. 1998. Bayesian analysis of twinning and ovulation rates using a multiple trait threshold model and Gibbs sampling. J. Anim. Sci. 76:2048–2061.

Wallace, J. M., D. A. Bourke, P. Da Silva, and R. P. Aitken. 2001. Nutrient partitioning during adolescent pregnancy. Reproduction 122:347–357.

Wallace, J. M., T. R. H. Regnault, S. W. Limesand, W. W. Hay Jr., and R. V. Anthony. 2005. Investigating the causes of low birth weight in contrasting ovine paradigms. J. Physiol. 565:19–26.

Wilkes, P. R., I. B. Munro, and W. V. Wijeratne. 1978. Studies on a sheep freemartin. Vet. Rec. 102:140–142.

Wright, S. 1934. An analysis of variability in number of digits in an inbred strain of Guinea pigs. Genetics 19:506–536.

Wu, G., F. W. Bazer, J. M. Wallace, and T. E. Spencer. 2006. Intrauterine growth retardation: Implications for the animal sciences. J. Anim. Sci. 84:2316–2337.