Polwarth and Texel ewe parturition duration and its association with lamb birth asphyxia

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ABSTRACT: The objective of the present study was to test the hypothesis that parturition duration is related to birth asphyxia in lambs and that asphyxia affects newborn lamb viability and vigor. Two sire and dam genotypes (Texel: TX; Polwarth: PW) and their crosses were represented in the study. Eighty lambs (25 PW sire × PW dam, 13 TX × TX, 25 TX × PW, and 17 PW × TX) born to 69 grazing ewes were used. At birth, the log10 length of the second stage of parturition, birth weight, placental weight, and several body measurements were recorded on all lambs, and jugular blood samples were analyzed with the i-StatPortable Clinical Analyzer (Abbott, Montevideo, Uruguay). A modified Apgar viability score at birth and lamb behavior during their first hour of life were recorded. Brain weight, muscle:bone ratio, and bone density were recorded in 20 male lambs (5 from each breed group) that were euthanized and dissected 24 h after birth. Data were analyzed by linear regression, least squares ANOVA, and ordinal and binary logistic regressions. Mean blood gas and acid-base variables were 7.21 ± 0.09 for pH, 18.4 ± 9.8 mmHg for partial pressure of oxygen, 53 ± 12.5 mmHg for partial pressure of carbon dioxide, and −4 ± 5.1 mmol/L for extracellular fluid base excess. Parturition duration increased with birth weight (P < 0.001) and was shorter in TX ewes (P < 0.001), female lambs (P < 0.05), twins (P < 0.09), and twin females (sex × litter size interaction, P < 0.02). Twenty-six (32.9%) lambs were born asphyxiated (pO2 < 10 mmHg or pH < 7.1). Parturition duration increased the risk of asphyxia (P < 0.001), decreased the viability score (P < 0.001), and increased the latency to suckle the udder (P < 0.05). Twin-born lambs presented at birth a 16-fold greater risk of asphyxia (P < 0.01) and reduced placental efficiency (P < 0.05). Texel-sired lambs appeared immature at birth, with less bone density (P < 0.05), smaller brain (P < 0.05), shorter forelimbs (P < 0.05), greater anterior (P < 0.001) and posterior (P < 0.05) neck circumference, and greater muscle:bone ratio (P < 0.05). Immaturity may explain greater TX biotype survival. Together these results demonstrate that a relationship exists between parturition duration, neonatal viability and behavior, and acid-base balance values in single- and twin-born lambs, suggesting that birth asphyxia is an important risk factor in perinatal lamb mortality.

Key words: acid-base balance, birth asphyxia, parturition duration, Polwarth, sheep, Texel

INTRODUCTION

Perinatal lamb mortality is a serious problem biologically and economically for the sheep industry worldwide. Despite marked improvement in ewe nutrition, management, and health control, the increased mortality rate has not decreased significantly, and it remains as an intractable problem (Cloete and Scholtz, 1998). The reasons for this could be remaining unrecognized basic causes of lamb deaths. Dutra et al. (2007) showed that injury to the central nervous system could be an important factor in perinatal losses because lambs that died between birth and 5 d of age had neurologic lesions of hypoxic-ischemic encephalopathy of variable type and severity. The curve of perinatal mortality was typical of a point-source epidemic, suggesting intrapartum asphyxia as a major factor of lamb mortality. Reduced neck muscle mass and a slender skeletal structure of dead lambs suggested that body conformation is important for parturition survival (Dutra et al., 2007).

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Fetal asphyxia, defined as a condition of impaired blood gas exchange leading to progressive hypoxemia and hypercapnia with a significant metabolic acidosis (Low, 1996), is a common cause of morbidity and mortality in human newborns (Volpe, 2001), piglets (Randall, 1971; Herpin et al., 1996), calves (Bleul et al., 2008), and foals (Vaala, 1999). Prolonged parturition affects newborn lamb ability to stand and seek the teat (Dwyer et al., 1996) and is associated with increased perinatal lamb mortality in twin and triplet lambs (Everett-Hincks et al., 2007). However, birth asphyxia has not been investigated in newborn lambs, probably because acid-base balance must be measured soon after blood collection, which is not possible on farms located far from specialized clinical laboratories (Peiró et al., 2010). The objective of this study was to use a portable blood gas analyzer to evaluate the relationship between parturition duration to birth asphyxia in lambs and the impact of asphyxia on newborn viability and behavior.

**MATERIALS AND METHODS**

The experiment was carried out according to recommendations set by the Uruguayan Honorary Committee for Animal Ethics. It was conducted at the Experimental Unit “La Estanzuela” of INIA, Uruguay (35°S) in September 2007. The experimental design was a 2 × 2 factorial using 2 contrasting breeds: Polwarth (PW), representing wool-type, and Texel (TX), representing meat-type, with their crosses as mothers and sires.

**Animals**

Sixty-nine multiparous ewes grazing improved pastures as a single flock were used at 145 d of gestation. Body condition score (scale 1 to 5; Russell et al., 1969) and BW were recorded 1 wk before parturition. Polwarth ewes (n = 44, 59.4 ± 8.1 kg, 3.1 ± 0.5 BCS) were bred to either PW or TX rams (PW-PW, n = 20, TX-PW, n = 24). Texel ewes (n = 25, 82.9 ± 9.5 kg, 3.7 ± 0.6 BCS) were also bred to PW or TX rams (PW-TX, n = 14; TX-TX, n = 11). All ewes were acclimated to human presence before parturition until approach without disturbance.

**Data Recording**

The ewes were observed 24 h per day immediately before and during parturition. Length of second stage of parturition (appearance of fetal front or rear legs to complete lamb expulsion) was measured in all ewes. In twins, the birth of the first lamb was regarded as the beginning of parturition for the second lamb. Lambing assistance was given if the ewe failed to progress through parturition and 1) no appearance of lamb parts 1 h after the appearance of fluids, or 2) 2 h without lamb observation or evidence of malpresentation.

Fetal jugular blood (1 mL) was collected in the period of apnea (i.e., before the onset of regular respiratory movements) into heparinized (60 units) syringes to measure pH, partial pressure of oxygen (pO₂), oxygen saturation (saO₂), partial pressure of carbon dioxide (pCO₂), bicarbonate (HCO₃−), total CO₂ (tCO₂), and extracellular fluid base excess (BEₑcf), using the EG7+ cartridge of the i-STAT Portable Clinical Analyzer (Abbott, Montevideo, Uruguay). Jugular vein sampling was preferred because it is more reliable than umbilical cord sampling under field conditions and better reflects the fetal acid-base status at the time of birth (Westgate et al., 1994). The i-STAT has been validated for human patients (Papadea et al., 2002), cats and dogs (Groenbaugh et al., 1998; Verwaerde et al., 2002), newborn piglets (van Dijk et al., 2006), cattle, horses, and sheep (Peiró et al., 2010), and in experimental fetal lamb models (Houfflin-Debarge et al., 2005; Acharya et al., 2008). Samples were analyzed within 2 min of collection, and no correction for body temperature was made. Blood glucose was measured using a single drop with the Medisense2 blood glucose meter (Medisense Inc., Bedford, MA).

The viability at birth was measured at 1 or 5 min, or both, of life using a modified Apgar scoring system formulated by the authors (Table 1). Apgar (mnemonic for the following signs: appearance, pulse, grimace, activity, and respiration) is a simple, reliable scoring system for evaluating the health of babies immediately after birth (American Academy of Pediatrics et al., 2006). Comparable Apgar scoring systems have been used to evaluate newborn piglets, foals, and puppies (Randall, 1971; Vaala, 1999; Veronesi et al., 2009). For human babies, a 5-min Apgar score of 7 to 10 is considered normal; scores of 4, 5, and 6 are intermediate and might require some resuscitative measures; and a score of 0 to 3 at 5 min requires immediate resuscitation because it is associated with increased risk of hypoxic ischemic encephalopathy (American Academy of Pediatrics et al., 2006). No resuscitation measures were applied in this study. Lamb behavior was measured during the first hour of birth, including the time of first attempt to stand and suckle, time of first success in standing and suckling, and how much time the lamb spent suckling.

One hour after birth, all lambs were weighed, ear tagged, and sexed, and data were recorded. The anterior and posterior neck circumference, circumference of the head and thorax, crown to rump length, width of the forehead and breast, and height of the front legs were measured. The relative birth BW (BW of lamb as percentage of ewe BW) for each lamb was calculated.

Ewe observation continued until placenta expulsion, at which time total placental weight and number and weight of cotyledons were recorded. Only the entire placentas were collected. The efficiency of the cotyledonary mass was calculated as grams of lamb born per gram of cotyledons. In the few cases when both twin fetuses were expelled separately from both placentas,
Carcass Composition, Brain Size, and Skeletal Maturity

Twenty lambs (5 from each breed group) were euthanized 24 h after birth by intravenous administration of sodium pentobarbitone in excess. Selected lambs were single-born males with normal parturition and suckling. Immediately after euthanasia, the whole brain was extracted intact and weighed. The entire neck (C1 to C7), right hind limb, and thoracolumbar spine (T10 to L7) were removed from each lamb, sealed in plastic bags, and stored frozen at −20°C before analysis. Samples were then thawed and dissected into muscle, bone, and soft tissue. Each tissue was weighed, and muscle:bone ratio was calculated. The right talus, right femur, and second lumbar vertebra (L2), representing an early-, intermediate-, and late-maturing bone, respectively (Butterfield, 1988), were cleaned of soft tissue and weighed in air and then in 95% ethanol. Their density was determined based on Archimedes’ principle of buoyancy, using the following formula: weight of the bone in air / (weight of the bone in air × weight of the bone in 95% ethanol) × 0.78. Ethanol, 95% (measured density: 0.78 g/mL), was chosen because many small bones floated in water. Bone density (g/mL) was considered as an index of skeletal maturity.

Statistical Analysis

Data were analyzed by ANOVA using the GLM procedure (Minitab Inc., 2005, State College, PA). Parturition duration and lamb behavior data were log10 transformed because they were not normally distributed. Fixed factors in all models were sire and dam breed (PW, TX), lamb sex (male, female), and litter size (single, twin), except the model for carcass composition that included sire and dam breed only. Two-way interactions were included only when preliminary analysis indicated significance (P < 0.05). Parturition duration and lamb body dimensions were analyzed with birth weight as a covariate, acid-base variables were analyzed with birth weight, placental weight, and log10 parturition duration as covariates, whereas birth weight and log10 parturition duration were fitted as covariates to analyze lamb behavior. Multiple comparison tests within the factors were performed using Tukey’s significant difference test. To analyze differences in bone density among anatomical regions, a general linear model with lamb as a fixed factor was used. The Kaplan-Meier survival function was used to estimate the median time to each lamb behavior trait.

Ordinal and binary logistic regressions were used to analyze Apgar score and the severity of asphyxia, respectively. Apgar score was subdivided into 3 viability groups (Apgar 0 to 3 = low viability, Apgar 4 to 6 = medium viability, and Apgar 7 to 10 = high viability lambs), whereas lambs with acidosis (pH ≤ 7.1) or hypoxia (pO2 ≤ 10 mmHg), or both, were assigned to the asphyxiated group (Helwig et al., 1996). Sire and dam breed, lamb sex, and litter size were fitted as fixed factors in both models, with birth weight, placental weight, and log10 parturition duration fitted as covariates.

Correlation coefficients and linear regression analysis were used to measure the degree of relationship between continuous variables. Frequency data were analyzed with χ2 test of association. The data are presented as mean ± SD, median and percentiles, or least squares means ± SEM. Probability values ≤0.05 were considered significant.

RESULTS

Descriptive Statistics

In total, 80 lambs (PW × PW, n = 25; TX × PW, n = 25; TX × TX, n = 13; and PW × TX, n = 17; sire breed × dam breed) were born. There were 56 singles and 24 twin-born lambs, and 40 were males and 40 females. Twenty-one lambs (26.2%) were assisted at birth, with more PW (P < 0.05) than TX ewes (χ2 = 6.547; 36 vs. 10%). One TX × PW twin male died within 1 min of birth and was cyanotic with Apgar 0, pH 7.063, pCO2 48.9 mmHg, pO2 15 mmHg, and BEecf −16 mmol/L, indicating asphyxia with severe metabolic acidosis (Low, 1996). Postmortem examination of this lamb revealed neurologic lesions of hypoxic-ischemic encephalopathy, characterized by edematous and congested brain, multiple hemorrhages in the leptomeninges, and numerous microhemorrhages in the medulla oblongata and cervical cord, identical to those described previously for intrapartum death in lambs (Dutra et al., 2007). Because of missing data (blood
Table 2. Jugular blood values for acid-base variables, blood gases, and glucose of lambs at birth

<table>
<thead>
<tr>
<th>Variable</th>
<th>n²</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
<th>5th percentile</th>
<th>Median</th>
<th>95th percentile</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>79</td>
<td>7.21</td>
<td>0.09</td>
<td>6.99 to 7.41</td>
<td>7.05</td>
<td>7.23</td>
<td>7.36</td>
</tr>
<tr>
<td>BE_{ecf}, mmol/L</td>
<td>79</td>
<td>−1.29</td>
<td>5.1</td>
<td>(−16) to (9)</td>
<td>−9.74</td>
<td>−1.00</td>
<td>7.16</td>
</tr>
<tr>
<td>pO₂, mmHg</td>
<td>78</td>
<td>18.4</td>
<td>9.8</td>
<td>4 to 53</td>
<td>0</td>
<td>18.0</td>
<td>34.5</td>
</tr>
<tr>
<td>saO₂, %</td>
<td>78</td>
<td>21.2</td>
<td>16.6</td>
<td>0 to 85</td>
<td>0</td>
<td>19.0</td>
<td>48.6</td>
</tr>
<tr>
<td>pCO₂, mmHg</td>
<td>79</td>
<td>65.4</td>
<td>12.5</td>
<td>29.6 to 103.7</td>
<td>44.8</td>
<td>63.7</td>
<td>86.1</td>
</tr>
<tr>
<td>tCO₂, mmol/L</td>
<td>79</td>
<td>28.53</td>
<td>4.1</td>
<td>15 to 38</td>
<td>21.6</td>
<td>29.0</td>
<td>35.3</td>
</tr>
<tr>
<td>HCO₃⁻, mmol/L</td>
<td>79</td>
<td>26.5</td>
<td>4.0</td>
<td>13.9 to 35.4</td>
<td>19.5</td>
<td>26.8</td>
<td>33.1</td>
</tr>
<tr>
<td>Glucose, mg/dL</td>
<td>78</td>
<td>53.1</td>
<td>23.4</td>
<td>18 to 109</td>
<td>14.5</td>
<td>45.5</td>
<td>91.7</td>
</tr>
</tbody>
</table>

1BE_{ecf} = extracellular fluid base excess (mmol/L); pO₂ = partial pressure of oxygen (mmHg); saO₂ = oxygen saturation; pCO₂ = partial pressure of carbon dioxide (mmHg); tCO₂ = total carbon dioxide (mmol/L); HCO₃⁻ = bicarbonate concentration (mmol/L).

2Missing values are due to blood clotting in 1 lamb and incomplete data recording (pO₂ and saO₂) in another.

Correlation Among Blood Gases, Acid-Base Variables, and Blood Glucose

Blood gases, acid-base variables, and blood glucose concentration showed considerable variation and correlation at birth. Blood pO₂ ranged from 4.0 to 53.0 mmHg, saO₂ from 0 to 85%, pCO₂ from 29 to 103 mmHg, BE_{ecf} from −16 to 9.0, and pH from 6.99 to 7.42 at the time of birth. Although the data were slightly skewed, the mean and median values for pH, pCO₂, pO₂, and BE_{ecf} were similar (Table 2).

Blood pH showed a negative (P < 0.001) linear correlation with pCO₂ (r = −0.69) and a positive (P < 0.001) correlation with BE_{ecf} (r = 0.79). Glucose concentration ranged between 18 and 109 mg/dL and showed a linear (P < 0.001) relationship with the extent of asphyxia, decreasing with blood pH (r = −0.456) and BE_{ecf} (r = −0.362), and increasing with blood pCO₂ (r = 0.31).

Factors Affecting Acid-Base Variables and Blood Gases at Birth

Results of the model used to analyze factors affecting pH, pCO₂, pO₂, HCO₃⁻, BE_{ecf}, and glucose values in jugular vein blood at birth are shown in Table 3. Litter size was found to influence most acid-base balance variables. At birth, twin-born vs. single lambs had decreased blood pH (7.17 vs. 7.23, P < 0.05), BE_{ecf} (−4.04 vs. 0.05 mmol/L, P = 0.01), and HCO₃⁻ (24.5 vs. 27.3 mmol/L, P < 0.05), and a greater concentration of tCO₂ (29.3 vs. 26.6 mmol/L, P < 0.05). Oxygenation was numerically smaller as well (15.9 vs. 19.5 mmHg, P > 0.05).

Blood pO₂, BE_{ecf}, and HCO₃⁻ values were affected by parturition duration and placental weight. An increasing parturition duration resulted in a decrease in pO₂ (regression coefficient = −13.09, P < 0.001), BE_{ecf} (regression coefficient = −3.93, P < 0.05), and HCO₃⁻ (regression coefficient = −3.27, P < 0.05). On the other hand, fetal blood oxygenation increased with increasing placental weight (regression coefficient = 0.018, P < 0.05). An interaction for oxygenation was found between breed types, with TX × PW lambs having decreased blood pO₂ compared with purebred PW lambs (14.0 vs. 20.8 mmHg, P < 0.008).

Blood glucose was affected by dam breed, with lambs born to PW ewes showing greater (P < 0.01) glucose concentration at birth than lambs from TX ewes (59.7 vs. 41.4 mg/dL, respectively).

Factors Affecting Probability of Birth Asphyxia

Seventeen lambs (21.2%) had a pO₂ blood value of <10 mmHg indicative of severe birth hypoxemia (Robinson et al., 1977; Gardner et al., 2002). When a value of pH ≤7.1 was used to define fetal acidosis (Helwig et al., 1996), pCO₂ ≥ 70 mmHg to define respiratory acidosis (Helwig et al., 1996), and a base excess of −10 mmol/L or less was regarded as indicative of pathologic metabolic acidosis (Low, 1996), 10 lambs (12.5%) were acidic: 2 with pure metabolic acidosis (pH ≤ 7.1 and pCO₂ ≥ 70 mmHg), and 8 with mixed acidosis (pH ≤ 7.1, pCO₂ ≥ 70 mmHg, and BE_{ecf} ≤ −10 mmol/L).

None had pure respiratory acidosis (i.e., pH ≤ 7.1, BE_{ecf} ≥ −10 mmol/L, and pCO₂ ≥ 70 mmHg). In total, 26 (32.9%) lambs were hypoxicemic or acidic, or both, at birth and were assigned to the asphyxiated group for logistic regression analysis.

Probability of asphyxia was affected by parturition duration, dam breed, and litter size (Table 4). Parturition duration positively influenced (P = 0.001) asphyxia probability (estimated coefficient 4.51 ± 1.37, z = 3.27), with the overall asphyxia risk increasing 90.9 times for each supplementary 10 min elapsed (Figure 1). Lambs born to TX ewes had a 9% probability (0.09 odds ratio, P = 0.01, Table 4) of being born asphyxiated compared with lambs born to PW ewes. Risk of asphyxia was 15.6 times greater in twin- than in single-born lambs (P = 0.01). Birth asphyxia was not affected by birth weight, placental development, lamb sex, or sire breed.
Table 3. Effect of ewe and ram breed, litter size, lamb sex, and birth weight on acid-base balance and blood gases in lambs at birth \(^1\)

<table>
<thead>
<tr>
<th>Variable</th>
<th>pH (n = 68)</th>
<th>pCO2 (n = 68)</th>
<th>BE ecf (n = 67)</th>
<th>pO2 (n = 67)</th>
<th>Glucose (n = 66)</th>
<th>HCO3(^–) (n = 67)</th>
<th>tCO2 (n = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ewe breed (^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PW</td>
<td>7.19</td>
<td>67.9</td>
<td>−1.97</td>
<td>17.4</td>
<td>59.7(^a)</td>
<td>26.1</td>
<td>NS</td>
</tr>
<tr>
<td>TX</td>
<td>7.21</td>
<td>62.9</td>
<td>−2.01</td>
<td>18.1</td>
<td>41.4(^b)</td>
<td>25.7</td>
<td>NS</td>
</tr>
<tr>
<td>Ram breed (^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PW</td>
<td>7.20</td>
<td>68.7</td>
<td>−1.63</td>
<td>18.1</td>
<td>52.6</td>
<td>26.2</td>
<td>NS</td>
</tr>
<tr>
<td>TX</td>
<td>7.20</td>
<td>62.1</td>
<td>−2.35</td>
<td>17.4</td>
<td>48.5</td>
<td>25.7</td>
<td>NS</td>
</tr>
<tr>
<td>Ewe × ram breed (^2)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PW × PW</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>0.008</td>
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<td>NS</td>
</tr>
<tr>
<td>PW × TX</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>20.8(^a)</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>TX × PW</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>14.0(^b)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>TX × TX</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>15.4(^ab)</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Lamb sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>7.20</td>
<td>65.2</td>
<td>−1.31</td>
<td>16.3</td>
<td>50.0</td>
<td>26.6</td>
<td>27.3</td>
</tr>
<tr>
<td>Female</td>
<td>7.20</td>
<td>65.6</td>
<td>−2.67</td>
<td>18.8</td>
<td>51.1</td>
<td>25.3</td>
<td>28.8</td>
</tr>
<tr>
<td>Litter size</td>
<td></td>
<td>0.023</td>
<td>0.009</td>
<td></td>
<td></td>
<td></td>
<td>0.017</td>
</tr>
<tr>
<td>Single</td>
<td>7.23(^a)</td>
<td>67.8</td>
<td>0.05(^a)</td>
<td>19.5</td>
<td>51.8</td>
<td>27.3(^a)</td>
<td>26.6(^c)</td>
</tr>
<tr>
<td>Twin</td>
<td>7.16(^b)</td>
<td>63.0</td>
<td>−4.04(^b)</td>
<td>15.9</td>
<td>49.3</td>
<td>24.3(^b)</td>
<td>29.3(^c)</td>
</tr>
<tr>
<td>Covariates</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>0.018</td>
<td>NS</td>
<td>1.29</td>
<td>1.57</td>
<td>NS</td>
<td>−2.09</td>
<td>NS</td>
</tr>
<tr>
<td>Parturition duration, log(_{10}) min</td>
<td>−0.004</td>
<td>NS</td>
<td>−0.75</td>
<td>−3.93</td>
<td>0.025</td>
<td>−13.09</td>
<td>NS</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>−0.000</td>
<td>NS</td>
<td>0.007</td>
<td>−0.002</td>
<td>NS</td>
<td>0.018</td>
<td>0.023</td>
</tr>
</tbody>
</table>

\(^a,b\)Least squares means with different superscripts in the same column and within a variable differ.

\(^1\)pCO2 = partial pressure of carbon dioxide (mmHg); BE ecf = extracellular fluid base excess (mmol/L); pO2 = partial pressure of oxygen (mmHg); HCO3\(^–\) = bicarbonate concentration (mmol/L); tCO2 = total carbon dioxide (mmol/L); glucose concentration (mg/dL); LSM = least squares means.

\(^2\)TX = Texel; PW = Polwarth; sire breed is listed first.

\(^3\)NS = not significant.
Factors Affecting Parturition Duration and Fetal Membrane Development

Duration of parturition was affected by birth weight, with the parturition duration increasing 10 min for each supplementary 141 g of birth weight (regression coefficient = 0.141 ± 0.043, \( P < 0.001 \)). The adjusted \( R^2 \) value showed that the model fits the data rather well, explaining 55.11% of the parturition duration variance (data not shown).

Texel ewes had a shorter (\( P < 0.001 \)) parturition duration than PW ewes (13.5 ± 1.14 vs. 21.9 ± 1.12 min, respectively). In contrast, TX-sired lambs had longer (\( P < 0.05 \)) parturition duration than lambs sired by PW rams (21.4 ± 1.14 vs. 13.8 ± 1.12 min, respectively). These effects were additive, and hence parturition duration was shortest (\( P < 0.05 \)) in PW × TX lambs compared with other sire × dam combinations (9 ± 1.19 vs. > 20 ± 1.17 min, respectively). Parturition duration was also shorter (\( P < 0.05 \)) for female lambs than for male lambs (14.7 ± 1.16 vs. 20.1 ± 1.11 min, respectively), numerically less in twins vs. singles (14.7 ± 1.17 vs. 20.1 ± 1.11 min, \( P = 0.09 \)), and a sex × litter size interaction (\( P < 0.05 \)) resulted in twin female lambs having the shortest parturition duration (10.2 ± 1.25 vs. > 19 ± 1.21 min). Twin-born lambs had fewer (\( P < 0.001 \)) cotyledons (53 ± 3.5 vs. 67 ± 2.3) but greater (\( P < 0.05 \)) total weight of fetal membranes (602.7 ± 31.9 vs. 529.7 ± 21.0 g), total cotyledon weight (227.6 ± 14.9 vs. 189.4 ± 9.8 g), and mean cotyledon weight (4.29 ± 0.27 vs. 2.92 ± 0.17 g) than single-born lambs, respectively.

Factors Affecting Lamb Dimensions, Body Composition, and Brain Size at Birth

Lamb birth weight, expressed as a percentage of ewe BW, was greater (\( P < 0.001 \)) in single-born (8 vs. 6%), TX-sired (7.4 vs. 6.6%), and lambs born to PW ewes (7.7 vs. 6.3%) compared with twin-born, PX-sired, and lambs born to TX ewes, respectively. These effects were additive, and hence, PW ewes sired by TX rams carried relatively larger lambs than the other dam-sire combinations, explaining their greater birth assistance (52 vs. <23%, \( P < 0.001 \)).

Texel-sired lambs had greater anterior (\( P < 0.001 \)) neck circumference (20.3 ± 0.2 vs. 19.0 ± 0.2 cm), posterior (\( P < 0.05 \)) neck circumference (22.6 ± 0.2 vs. 21.3 ± 0.2 cm), and shorter (\( P < 0.05 \)) forelimb height (24.9 ± 0.2 vs. 25.7 ± 0.2 cm), when compared with PW-sired lambs at equal birth weight. These TX-sired lambs were more muscular with greater (\( P < 0.05 \)) muscle:bone ratio in the neck (1.97 ± 0.15 vs. 1.63 ± 0.19), thoracolumbar (1.34 ± 0.13 vs. 0.99 ± 0.18), and hind limb regions (2.10 ± 0.08 vs. 1.76 ± 0.10) than PW-sired lambs, respectively. However, only hind limb region remained significant after correcting for birth weight. There was a centripetal bone density gradient (\( P < 0.001 \)) across the skeleton, with density being greater (\( P < 0.05 \)) in talus than femur (1.105 ± 0.005 vs. 1.121 ± 0.007 g/mL, \( P < 0.05 \)) and L2 vertebra (1.121 ± 0.007 vs. 1.107 ± 0.004 g/mL). Mean density of all bones measured tended to be less in TX- than PW-sired lambs, which was statistically apparent for L2 vertebra after correction for birth weight (1.105 ± 0.005 vs. 1.121 ± 0.007 g/mL, \( P < 0.05 \)). Birth weight-adjusted brain size was greater (\( P < 0.05 \)) in both PW-sired lambs (63.0 ±

**Table 4.** Binary logistic regression table with variables related to the log odds of a lamb being born asphyxiated (\( n = 69 \))

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient (±SE) (^1)</th>
<th>z</th>
<th>Odds ratio (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−11.64 ± 3.58</td>
<td>−3.25</td>
<td>—</td>
<td>0.002</td>
</tr>
<tr>
<td>TX sire</td>
<td>0.475 ± 0.821</td>
<td>0.58</td>
<td>1.61 (0.32 to 8.034)</td>
<td>NS (^2)</td>
</tr>
<tr>
<td>TX dam</td>
<td>2.355 ± 0.961</td>
<td>2.45</td>
<td>0.09 (0.01 to 0.63)</td>
<td>0.01</td>
</tr>
<tr>
<td>Female lamb</td>
<td>1.276 ± 0.773</td>
<td>1.65</td>
<td>3.58 (0.79 to 16.32)</td>
<td>NS</td>
</tr>
<tr>
<td>Twin-born</td>
<td>2.752 ± 1.077</td>
<td>2.55</td>
<td>15.6 (1.9 to 129.6)</td>
<td>0.01</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>0.427 ± 0.55</td>
<td>0.77</td>
<td>1.53 (0.52 to 4.32)</td>
<td>NS</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>−0.061 ± 0.002</td>
<td>−0.57</td>
<td>1 (0.99 to 1)</td>
<td>NS</td>
</tr>
<tr>
<td>Parturition duration, log(_{10}) min</td>
<td>4.510 ± 1.378</td>
<td>3.27</td>
<td>90.9 (6.09 to 1,356.7)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

\(^1\)The estimated coefficient of a predictor (factor or covariate) is the estimated change in the probability log of asphyxiated/probability log of not asphyxiated for each unit change in the predictor, assuming the other predictors remained constant.

\(^2\)TX = Texel.

\(^3\)NS = not significant.

**Figure 1.** Effect of parturition duration (log\(_{10}\) min) on the probability of birth asphyxia in lambs.
1.15 vs. 59.6 ± 1.06 g) and in lambs born to PW ewes (63.1 ± 1.05 vs. 59.5 ± 1.14 g). Brain weight relative to birth weight was greater (P < 0.01) in PW-sired lambs than in their TX-sired counterparts (1.22% ± 0.04 vs. 1.05% ± 0.04).

Factors Affecting Lamb Viability and Behavior

Four (5%) newborn lambs were of low viability (Apgar 0 to 3), 18 (22.5%) lambs were of medium viability (Apgar 4 to 6), and 58 (72.5%) lambs were of high viability (Apgar 7 to 10). Predictors related (P < 0.01) to the log odds of a lamb being born with smaller Apgar score were parturition duration and litter size (Table 5). Parturition duration increased (P < 0.01) linearly the risk of poor viability at birth (estimated coefficient = 4.061 ± 1.18, Table 5), with odds of low vs. medium and medium vs. high viability score increasing 58 times for each supplementary 10-min elapsed time (Table 5). The odds ratio indicated that twin-born lambs had a 7-fold greater probability of being born with decreased viability score than singletons (odds ratio = 6.95, P < 0.05).

The estimated median time for first attempt to stand and suckle and first success in standing and suckling was 12, 18, 20, and 34 min, respectively. Approximately 85% of all lambs made standing attempts in 25 min, stood in 36 min, made suckle attempts in 37 min, and sucked in 54 min. At 140 min postpartum, all lambs had sucked. Latency between first attempt to suckle and first success in suckling was longer (P < 0.01) in lambs born to TX ewes (21.5 ± 1.16 min) vs. PW ewes (12.8 ± 1.15 min), and in twin-born (22.0 ± 1.20 min) vs. single-born (12.5 ± 1.12 min) lambs, and it increased (P < 0.05) with an increasing parturition duration (Table 5). When BE_{ecf} and pO₂ blood values were fitted as covariates in the model, instead of parturition log₁₀ length, the latency to suckle the udder increased with decreasing pO₂ (regression coefficient = −0.0116, P < 0.001) and BE_{ecf} (regression coefficient = −0.019, P < 0.05), indicating that the effect of parturition duration was mediated through hypoxemia or metabolic acidosis, or both.

**DISCUSSION**

This experiment demonstrated a relationship between parturition duration and acid-base balance values at birth in newborn lambs. It was shown that prolonged parturition causes hypoxia, hypercapnia, and metabolic acidosis, which can produce intrapartum death due to hypoxic ischemic encephalopathy, reduce lamb viability at birth, and adversely affect postnatal vitality. Furthermore, many of the well-known prenatal risk factors for lamb mortality analyzed in this study (lamb birth weight, placental size, litter size, lamb sex, lamb body dimensions, sire, and dam breed) had an effect on parturition duration or hypoxia/acidosis that ensue, suggesting that birth asphyxia could be a key factor in perinatal lamb mortality.

Published values for pH and other components of the acid-base state in lambs born under grazing conditions are not available. In the present study, mean values and SD in the jugular vein at birth were 7.21 ± 0.09 for pH, 18.4 ± 9.8 mmHg for pO₂, 53 ± 12.5 mmHg for pCO₂, and −4 ± 5.1 mmol/L for BE_{ecf}. The normal blood values reported in experimental fetal sheep models before parturition onset are pH > 7.35, pO₂ > 25 mmHg, pCO₂ < 50 mmHg, and BE_{ecf} > −2.5 mmol/L (Comline and Silver, 1972; Nijland et al., 1995; Gardner et al., 2002). Major changes occur 15 min before delivery, when pO₂ and pH begin to decline and pCO₂ increases throughout expulsion, with mean values at birth comparable with this study (Comline and Silver, 1972). Similar mean values for pH and other components of the acid-base state have been reported in newborn piglets (Randall, 1971; Herpin et al., 1996; van Dijk et al., 2006), calves

**Table 5.** Ordinal logistic regression table with predictors related to the log odds of a lamb being born with decreased vitality (n = 69)

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Coefficient (±SE)</th>
<th>z</th>
<th>Odds ratio</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Const (1)</td>
<td>−9.20 ± 2.80</td>
<td>−3.28</td>
<td>—</td>
<td>***</td>
</tr>
<tr>
<td>Const (2)</td>
<td>−6.76 ± 2.65</td>
<td>−2.54</td>
<td>—</td>
<td>**</td>
</tr>
<tr>
<td>TX sire³</td>
<td>1.19 ± 0.80</td>
<td>1.48</td>
<td>3.29</td>
<td>NS⁴</td>
</tr>
<tr>
<td>TX dam³</td>
<td>−0.09 ± 0.79</td>
<td>−0.12</td>
<td>0.91</td>
<td>NS</td>
</tr>
<tr>
<td>Female lamb</td>
<td>0.78 ± 0.68</td>
<td>1.14</td>
<td>2.19</td>
<td>NS</td>
</tr>
<tr>
<td>Twin-born</td>
<td>1.93 ± 0.92</td>
<td>2.10</td>
<td>6.95</td>
<td>*</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>−0.53 ± 0.51</td>
<td>−1.05</td>
<td>0.58</td>
<td>NS</td>
</tr>
<tr>
<td>Placental weight, g</td>
<td>0.002 ± 0.002</td>
<td>0.88</td>
<td>1.00</td>
<td>NS</td>
</tr>
<tr>
<td>Parturition duration, log₁₀ min</td>
<td>4.061 ± 1.18</td>
<td>3.42</td>
<td>58.0</td>
<td>***</td>
</tr>
</tbody>
</table>

¹Const (1) and Const (2) are estimated intercepts for the logit of the cumulative probability of high and medium viability, respectively.

²The coefficients are the estimated change in the logit of the cumulative viability class probability when a variable is present and all other variables are held constant.

³TX = Texel.

⁴NS = not significant.

*P < 0.05, **P < 0.01, ***P < 0.001.
Severe asphyxia (hypoxemia: $pO_2 < 10$ mmHg or acidemia; pH < 7.10, or both; Helwig et al., 1996; Low, 1996; Gardner et al., 2002) was found in 32.9% of sampled newborn lambs. It was shown that the risk of asphyxia increased linearly with increasing parturition duration and that asphyxiated lambs have decreased Apgar score at birth and are slow to suckle the udder. The only dead lamb had severe metabolic acidosis ($BE_{ecf} = −16$ mmol/L) with neurologic lesions of acute hypoxic ischemic encephalopathy. In human babies (Low, 1996; Ross and Gala, 2002), piglets (van Dijk et al., 2006), and experimental sheep fetuses (Ni- jland et al., 1995; Ikeda et al., 1998), the threshold of decompensation at which neurological lesions and death begin to appear is approximately $BE_{ecf} ≤ −10$ mmol/L, a value concordant with our finding. The pathogenesis of asphyxia due to prolonged parturition in lambs is probably related to the reduction in uterine blood flow caused by intense uterine contractility due to peak concentrations of oxytocin (Comline and Silver, 1972). Several experiments in fetal sheep models have also showed that repetitive umbilical cord occlusion, mimicking cord compression during parturition, induces hypoxia and metabolic acidosis with various types of brain damage due to hypoxic ischemic encephalopathy (Bennet et al., 1999; Rocha et al., 2004). Premature umbilical cord rupture, or total cord occlusion with a halted blood supply to the fetus, has been recognized as a cause of stillborn piglets (Randall, 1971; van Dijk et al., 2006) and acidotic newborn calves (Szenci et al., 1988; Bleul et al., 2008). The umbilical cord of lambs is relatively short, only 7 to 10 cm or about 1/4 of the length of the fetus, and it usually ruptures prematurely when the fetal pelvis passes through the pelvic cavity of the dam (Roberts, 1986), but the significance of this fact was not investigated in this study.

Twin lambs in this study presented decreased $pO_2$, pH, $HCO_3^−$, and $BE_{ecf}$ values and greater tCO$_2$ at birth with a 16-fold greater risk of asphyxia than singletons. These differences were due to litter size itself, as they were independent of birth weight, parturition duration, placental weight, sex, and parental breed. Cotyledonary efficiency was also reduced, probably because the exchange surface area per fetus declined with litter size (Reynolds et al., 2010). Because the ewe placenta is virtually impermeable to lactic acid, an increasing metabolic acidemia (i.e., lactic acid from anaerobic metabolism), in combination with an insufficient placenta, usually develops in utero during late gestation in twin fetuses (Robinson et al., 1977; Sparks et al., 1982). Moreover, experimental evidence has shown that sustained or repeated hypoxemia in utero reduces fetal buffering capacity and renders twin fetuses less capable to survive to a new episode of acute hypoxemia such as that suffered during normal parturition (Gardner et al., 2002). It therefore seems that greater hypoxemia and metabolic acidosis of twin-born lambs at birth may be an explanation for a litter size effect on lamb survival after accounting for birth weight differences.

This study showed that TX ewes had a significantly shorter duration of the expulsive stage of parturition than PW ewes (≈13 vs. 22 min). In addition, lambs born to TX ewes had 11 times less risk of being born asphyxiated. These differences were due to dam breed itself because differences persist after adjustments for birth weight, litter size, lamb sex, and sire breed. When lamb birth weight was expressed as a percentage of ewe body weight at lambing, it appeared that lambewe BW ratio was significantly smaller in TX ewes (6.3 vs. 7.7%), suggesting that parturition is easier in this breed, probably because of their larger body size and pelvic proportion. Decreased blood glucose concentrations in their lambs also support a less stressful parturition in TX ewes because blood glucose and catecholamines secretion are closely related to the degree of asphyxia in sheep fetuses (Robinson et al., 1977). Ganzábal et al. (2007) found that lambs born to F$_1$ TX crossbred ewes had a 10% greater survival rate than pure PW, Ile de France × PW, and Milchschaf × PW ewe biotypes. The shorter labor of TX ewes must be genetically determined because flock management and environment were similar for both breeds in this experiment. Parturition duration was previously found to be repeatable and statistically different between sheep breeds or genetic lines within a breed, an effect attributed to increased pelvic area of highly fertile ewes (Knight et al., 1988; Cloete and Haughey, 1990; Cloete and Scholtz, 1998).

Large differences in body conformation were found between TX and PW lamb biotypes at equal birth weight. Texel-sired neonates had a smaller brain size and were more compact and muscled, with shorter fore limbs, thicker necks, and greater muscle:bone ratios around the vertebral column and in the hind limbs. They appeared skeletally immature also, as revealed by the decreased bone density of L2 vertebrae. These anatomical differences may reflect differences in the stage of maturity at birth because TX, being of a larger mature size, is more immature than PW when compared at the same birth weight (Butterfield, 1988). Evaluation of TX as a terminal sire breed has shown that TX-sired progeny display greater survival rate and a more compact carcass shape when compared with other ram breeds (O’Ferrall, 1974; Leymaster and Jenkins, 1993). Their greater neck musculature may better stabilize the atlantoaxialis and atlanto-occipitalis joints during parturition, thus decreasing the risk of hemorrhages in the medulla oblongata and upper cervical spinal cord (Dutra et al., 2007). The shorter forelegs may account for the reduced incidence of shoulder-elbow flexion dystocia reported in purebred TX (Grommers et al., 1988), and foals (Vaala, 1999), and are comparable with population-based reference values of human infants (Westgate et al., 1994; Helwig et al., 1996). Thus, similar to other species, the parturition process in lambs is associated with biochemical signs of hypoxia and mild mixed respiratory-metabolic acidosis indicative of fetal asphyxia.
Brain size difference and, by association, brain development between TX (meat production breed) and PW (fine wool breed) neonates may be the result of divergent artificial selective breeding (Kruska, 2005). It is well established that survival time under hypoxia is inversely related to brain developmental maturity at birth (Bennet et al., 1999; Volpe, 2001) and that the fetal sheep brain of sheep fetuses has a greater metabolic rate and is more vulnerable to oxygen limitation than other organs (Ikeda et al., 1998; Bennet et al., 1999; Rocha et al., 2004). Therefore, a reduced oxygen demand of a smaller brain may allow TX progeny to better tolerate the normal asphyxia conditions that take place at birth.

Approximately 30% of the newborn lambs evaluated with the modified Apgar test in this experiment had a low or medium viability score at birth. These data coincide with the number of lambs that were born asphyxiated, indicating that the altered viability is a consequence of oxygen deprivation. The results also showed that prolonged parturition was more distressing for the twin-born lambs because they were 7-fold more likely to be born with a poorer viability score, independent of parturition duration, birth weight, sex, and breed. Intrapartum hypoxia may lead to central nervous system depression and damage the brain by a pathophysiologic process that progresses over many days (Volpe, 2001; Rocha et al., 2004). If the newborn lamb is born lethargic, it will take longer to shake the head and to achieve sternal recumbence (Dwyer et al., 1996), both important activities for establishment of the ewe-lamb bond (Alexander and Shillito, 1977). Similarly, parturition duration affected the behavior of the neonate in this study, principally the interval to first success in suckling the udder. Lambs must be sufficiently vigorous to suckle as soon as possible after birth, to be able to follow their dams when rejoining the flock, or when foraging (Alexander, 1958). Together with previous results, these findings suggest that intrapartum asphyxia reduces the capability of twin-born lamb to adapt to extrauterine life.

In conclusion, the present study showed that parturition duration and risk of asphyxia are closely related in lambs. Asphyxiation caused intrapartum death, decreased vitality score at birth, and affected the behavioral ability of the newborn lambs to seek the udder. The TX ewes had shorter parturition duration, and their lambs had a decreased risk of being born asphyxiated. From these results, it appears that identification of ewe and lamb factors affecting the expulsive stage of parturition and their associations with perinatal asphyxia are required for a better understanding and potential decrease of perinatal lamb mortality.

LITERATURE CITED


