Changes in fetal lamb arterial blood gas and acid-base status with advancing gestation

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Rurak D, Bessette NW. Changes in fetal lamb arterial blood gas and acid-base status with advancing gestation. Am J Physiol Regul Integr Comp Physiol 304: R908–R916, 2013. First published March 27, 2013; doi:10.1152/ajpregu.00430.2012.—To determine whether there are changes in blood gas and acid-base status with advancing gestation in the fetal lamb, similar to that reported in the human fetus, blood gas, acid-base, and blood metabolite values were measured in 447 control, arterial blood samples from 108 chronically instrumented fetal lambs between 103 and 146 days gestation. With advancing gestation, PO2, pH, O2 saturation, and O2 content fell significantly, while PCO2 and hemoglobin concentration increased. Blood glucose and lactate concentrations were unchanged, although the lactate level associated with decreased PO2 and glucose level and increased pH, multiple linear regression indicated that increasing fetal number was a limiting factor for fetal survival and development.

Fetal weight-normalized uterine and umbilical blood flows also decrease with advancing gestation in the sheep, horse, and guinea pig (30, 60). However, there is less information on gestational age-related changes in fetal blood gas and acid-base variables in these species. Bell et al. (7) have reported that fetal arterial O2 saturation in chronically catheterized fetal lambs at 75 days gestation is higher than in late gestation. In contrast, other studies of chronically instrumented fetal lambs and horses report no changes in fetal blood gas and acid-base variables with advancing gestation (12, 13, 16, 20, 24, 46). However, the sampling approaches used in these studies differed from those in the human cordocentesis studies mentioned above, in that either individual fetuses were sampled longitudinally over a relative short range of gestational ages (12, 13, 16) or small groups of fetuses were studied at different gestational age ranges (20, 24, 46). Given that in the fetal lamb, vascular PO2 and other blood gas and acid-base variables exhibit marked short-term fluctuations in association with fetal somatic activity and prelabor uterine contractions (27, 33, 43, 57, 71), it is unlikely that either of the experimental approaches used in the sheep and horse studies mentioned above could detect any gestational age-related changes in fetal blood gas and acid-base status.

In this article, we report blood-gas and acid-base variables measured in control samples or in control experiments from a large number of chronically instrumented fetal lambs from ~100 days of gestation to term in a number of different studies conducted in our laboratory over a 10-yr period. This allowed us to examine the changes in these variables over a range of gestation using an approach more similar to the human cordocentesis studies. We hypothesized that in the fetal lamb, blood gas and acid-base variables would change in relation to gestational age in a manner similar to that in the human fetus. In addition, because the pregnant sheep studied carried singleton, twins, or triplets, we also examined the effect of fetal number on the variables and their relationships to gestational age, given that in pregnant sheep, birth weight decreases with increasing fetal numbers (9). We hypothesized that the alterations in fetal blood gas and acid-base status with advancing gestational age would be greater in the twins and triplets. We also checked for sex differences in the measured variables.

OXYGEN IS ESSENTIAL FOR FETAL SURVIVAL AND NORMAL GROWTH AND DEVELOPMENT (26). Oxygen supply to the fetus is via placental transfer, and the efficiency of transfer is determined primarily by the magnitude and relative orientation of maternal and fetal placental blood flows (5). Because of the nature of placental oxygen transfer and the geometry of the fetal circulation, fetal vascular PO2 is much lower than after birth, and the fetus is, thus, physiologically hypoxic. However, because of several fetal adaptations (e.g., higher hemoglobin, higher O2 affinity of fetal hemoglobin or blood, higher relative cardiac output), oxygen delivery to most fetal organs and tissues is higher than in the adult (56). Thus, during a normal pregnancy, oxygen is likely not a limiting factor for fetal survival and development.

However, there is limited published information on the changes in vascular PO2 and other blood gas and acid-base variables during gestation. Much of the available data comes from human cross-sectional studies, in which blood gas and acid-base parameters were measured in umbilical arterial and venous blood samples obtained via cordocentesis from patients over a wide gestational age. The results indicate decreases in PO2 and O2 saturation and increases in PCO2 and hemoglobin concentration or hematocrit with advancing gestation (48, 50, 59, 62, 68). These alterations in fetal blood gas values may be the result of the progressive decline in uterine and umbilical blood flows when normalized to fetal weight with advancing gestational age (23, 37), since as mentioned above, the efficiency of placental O2 (and CO2) transfer is largely determined by the magnitudes of maternal and fetal placental blood flows.

Fetal weight-normalized uterine and umbilical blood flows also decrease with advancing gestation in the sheep, horse, and guinea pig (30, 60). However, there is less information on gestational age-related changes in fetal blood gas and acid-base variables in these species. Bell et al. (7) have reported that fetal arterial O2 saturation in chronically catheterized fetal lambs at 75 days gestation is higher than in late gestation. In contrast, other studies of chronically instrumented fetal lambs and horses report no changes in fetal blood gas and acid-base variables with advancing gestation (12, 13, 16, 20, 24, 46). However, the sampling approaches used in these studies differed from those in the human cordocentesis studies mentioned above, in that either individual fetuses were sampled longitudinally over a relative short range of gestational ages (12, 13, 16) or small groups of fetuses were studied at different gestational age ranges (20, 24, 46). Given that in the fetal lamb, vascular PO2 and other blood gas and acid-base variables exhibit marked short-term fluctuations in association with fetal somatic activity and prelabor uterine contractions (27, 33, 43, 57, 71), it is unlikely that either of the experimental approaches used in the sheep and horse studies mentioned above could detect any gestational age-related changes in fetal blood gas and acid-base status.

In this article, we report blood-gas and acid-base variables measured in control samples or in control experiments from a large number of chronically instrumented fetal lambs from ~100 days of gestation to term in a number of different studies conducted in our laboratory over a 10-yr period. This allowed us to examine the changes in these variables over a range of gestation using an approach more similar to the human cordocentesis studies. We hypothesized that in the fetal lamb, blood gas and acid-base variables would change in relation to gestational age in a manner similar to that in the human fetus. In addition, because the pregnant sheep studied carried singleton, twins, or triplets, we also examined the effect of fetal number on the variables and their relationships to gestational age, given that in pregnant sheep, birth weight decreases with increasing fetal numbers (9). We hypothesized that the alterations in fetal blood gas and acid-base status with advancing gestational age would be greater in the twins and triplets. We also checked for sex differences in the measured variables.

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METHODS

The studies reported in this article, in which the sheep (Suffolk, Dorset breeds) were used, were approved by the University of British Columbia Committee on Animal Care and conformed to the guidelines of the Canadian Council on Animal Care. The breeding method and surgical and experimental procedures employed on the animals have been previously described in our study of the effect of chronic fetal instrumentation on gestational length and birth weight in sheep (9). None of the data presented in this paper were obtained during the experiments conducted on these sheep.

Fetal blood gas, pH, glucose, and lactate measurements. Beginning on the day after surgery, fetal arterial blood samples (0.5 ml) were collected for measurement of blood gas, acid-base, and plasma metabolite concentrations. PO2, PCO2, pH, and base excess were measured with an IL 1306 (Allied Instrumentation Laboratory, Milan, Italy) or Radiometer ABL 500 (Radiometer, Copenhagen, Denmark) blood gas analyzer, while O2 saturation and hemoglobin concentration were determined with a Radiometer OSM3 oximeter. Blood glucose and lactate concentrations were measured with a Yellow Springs Instruments 2300 STAT (YSI, Yellow Springs, OH) or Radiometer 520 metabolite and electrolyte analyzer. The blood gas and metabolite results analyzed in this study were from samples obtained from the fourth day after surgery until the start of experimentation. If the experiment ended before delivery and if the condition of the fetus still appeared to be normal based upon blood gas values, heart rate, arterial pressure, and fetal breathing activity, the daily samples obtained after this time were included in the analysis.

Data analysis. The relationship between the fetal blood gas, acid-base and blood metabolite variable, and gestational age was analyzed by linear regression using the least squares method. The impact of fetal number and sex on these relationships was analyzed using multiple linear analyses, with the blood gas variables as the dependent variables, and gestational age, fetal number, and sex as the independent variables.

RESULTS

Blood gas, acid-base, and blood metabolite values were measured in 447 arterial blood samples from 108 chronically instrumented fetal lambs between 103 and 146 days gestation. There were 37 singletons (19 males, 18 females), 63 twins (36 males, 27 females), and 10 triplets (8 males, 2 females). The number of samples collected from an individual fetus ranged from 1 to 17 (mean = 4.1 ± 0.3) and the number of days over which sampling occurred was from 1 to 24 (mean = 5.8 ± 0.5) days. All operated fetuses survived until spontaneous delivery, although as reported previously (9), there was a significant incidence of preterm birth in the operated animals that was not present in the breeding flock from which the study animals were obtained. Birth weight averaged 3.84 ± 0.13, 3.40 ± 0.08, and 2.96 ± 0.23 kg for singletons, twins, and triplets, respectively.

Blood gas and acid-base status in relation to gestational age. Figure 1 gives plots of the relationship between gestational age and fetal arterial PO2, PCO2, pH, and base excess, while Fig. 2 gives the plots for oxygen saturation, hemoglobin concentration, and oxygen content. All of the relationships were statistically significant. PO2, pH, base excess, O2 saturation and content fell significantly, while Pco2 and hemoglobin concentration increased significantly with gestational age. The regression equation calculated for each variable was used to predict the values at 100 and 147 days. These values and the regression slopes ± SE are given in Table 1. Arterial PO2 decreased by 20.5%, while Pco2 increased by 13.1%. The % decrease in oxygen saturation (53.0%) was much greater than the fall in PO2, presumably because the decrease in pH and the increase in Pco2 would also decrease hemoglobin-oxygen affinity. Because of the increase in hemoglobin concentration,
the predicted fall in oxygen content between 100 and 147 (37.9%) was less than the decrease in oxygen saturation, but greater than the decrease in $PO_2$.

Figure 3 illustrates the changes in fetal arterial blood glucose and lactate concentrations with gestational age. Neither variable changed in relation to gestational age. However, a number of the samples had high lactate concentrations, particularly at gestational ages beyond ~125 days. Figure 4 demonstrates that the increased lactate concentrations were associated with fetal hypoxemia. Lactate concentration was inversely related both to arterial $PO_2$ and O$_2$ content, but there was a better fit of the data with $PO_2$. The relationship between lactate concentration and $PO_2$ was best described by an inverse third-order polynomial equation: $[\text{lactate}] = 188.1/PO_2 - 4.255.5/PO_2^2 + 32.855.6/PO_2^3 - 1.72$ ($r^2 = 0.4286$, $P < 0.001$). The analysis indicated that fetal arterial lactate concentration rose gradually as $PO_2$ decreased, with a much larger rate of rise with arterial $PO_2$ less than ~13 mmHg.

**Blood gas and acid-base status in relation to fetal number and sex.** These values in singleton, twin, and triplet fetuses are given in Table 2. Compared with singleton and twins, triplets were hypoxemic and had elevated lactic acid concentrations. In contrast, twins and triplets had significantly larger base excess values compared with singletons, and in twins, O$_2$ saturation decreased.

![Fig. 2](image2.png)  
Fig. 2. The relationship between gestational age and fetal arterial O$_2$ saturation (A), hemoglobin concentration (Hb; B) and blood O$_2$ content (C).

![Fig. 3](image3.png)  
Fig. 3. The relationship between gestational age and fetal arterial blood glucose (A) and lactate (B) concentrations.

### Table 1. Fetal arterial blood gas and acid-base values at 100 and 147 days gestation estimated from the regression equations given in Figure 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Slope ± SE, $P$</th>
<th>100 days</th>
<th>147 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>$PO_2$, mmHg</td>
<td>$-0.119 ± 0.036 (&lt;0.001)$</td>
<td>27.1</td>
<td>21.5</td>
</tr>
<tr>
<td>$PCO_2$, mmHg</td>
<td>$0.123 ± 0.032 (&lt;0.001)$</td>
<td>44.0</td>
<td>49.5</td>
</tr>
<tr>
<td>pH</td>
<td>$-0.155 ± 0.0003 (&lt;0.001)$</td>
<td>7.390</td>
<td>7.321</td>
</tr>
<tr>
<td>BE, meq/l</td>
<td>$-0.054 ± 0.019 (&lt;0.001)$</td>
<td>2.6</td>
<td>0.0</td>
</tr>
<tr>
<td>$SO_2$, %</td>
<td>$-0.922 ± 0.012 (&lt;0.001)$</td>
<td>81.8</td>
<td>38.5</td>
</tr>
<tr>
<td>Hb, g%</td>
<td>$0.065 ± 0.011 (&lt;0.001)$</td>
<td>8.7</td>
<td>11.7</td>
</tr>
<tr>
<td>$CO_2$, mM</td>
<td>$-0.037 ± 0.007 (&lt;0.001)$</td>
<td>4.6</td>
<td>2.9</td>
</tr>
</tbody>
</table>

BE, base excess.
was higher than in both singleton and triplets. In addition, there was a progressive decrease in blood glucose concentration with increasing fetal number. In comparing the values in male and female fetuses, the only significant difference was with hemoglobin concentration, with the value in female lambs (10.7 ± 0.2 mg%) being slightly but significantly higher than the male value (10.3 ± 0.1 mg%).

Table 3 gives the y intercept values and the regression coefficients for gestational age, fetal number, and sex that were estimated using multiple linear regression. Gestational age remained significantly related to PO₂, PCO₂, pH, base excess, oxygen saturation, hemoglobin concentration, and oxygen content. Fetal number had a negative impact on PO₂ and glucose concentration. The decrease in PO₂ with an increase in fetal number from singleton to twins or twins to triplets (0.94 ± 0.44 mmHg) is equivalent to the decrease over 4.8 days of gestation. In contrast, fetal number had a positive impact on pH, HCO₃⁻ base excess, and hemoglobin concentration, and for all variables, the regression coefficients were substantially larger than those for gestational age. The only sex difference was for hemoglobin concentration, with the female value being 0.52 ± 0.013 mg% higher than in males.

To determine whether there was a relationship between blood glucose concentration and birth weight, only data from the 46 animals that delivered at term (144.4 ± 0.3 day) were analyzed because including the fetuses that delivered preterm would introduce the confounding effect of prematurity on birth weight. The glucose samples used were those in the last sample collected prior to birth, and the time interval averaged 12.9 ± 0.9 days. As illustrated in Fig. 5, there was a significant positive relationship between fetal blood glucose concentration and birth weight; such that a 0.05-mM increase in glucose concentration would introduce the confounding effect of prematurity on birth weight.

Using the average blood glucose concentrations for singleton, twins, and triplets in Table 2, we used the regression equation in Fig. 5 to estimate the birth weights for each group. The estimated weights were 3.97, 3.80, and 3.54 kg for singleton, twins, and triplets, respectively. This compares to the actual average birth weights of 4.23, 3.60, and 3.07 kg for the fetuses born at term that were used in this analysis. There was no relationship between fetal arterial PO₂ and birth weight in this group.

DISCUSSION

The main finding of this study is that in pregnant sheep, fetal blood gas and acid-base status change with gestational age, as has been reported for the human fetus (48, 50, 59, 62, 68). The results also indicate that triplet fetal lambs are hypoxic and have elevated blood lactate concentrations compared with singleton and twin fetuses. In addition, there is a progressive decrease in fetal blood glucose concentrations with increasing fetal number, and for the animals born at term, a significant linear relationship can be made between blood glucose concentration and birth weight.

As reported previously by us (9) and Clark et al. (11), chronic instrumentation of fetal lambs is associated with a reduction in birth weight. This raises the question of whether fetal physiological variables, including blood gas measurements, obtained from such preparations are reflective of the values in normal, uninstrumented fetuses. The only studies that have attempted to address this question are those which have compared values measured in acutely anesthetized preparations, in which the fetus was either exteriorized or remained in utero, with values obtained from chronically instrumented lambs (2, 14). Both studies reported that the blood gas values in chronically instrumented fetuses were not different from those obtained from acutely anesthetized preparations, particularly in those in which the fetus remained within the uterus.

As noted in the introduction, a number of studies that have measured fetal blood gas and acid-base status in fetal lambs and horses over relatively short ranges of gestation or in small groups of animals at different times in gestation have not reported any gestational age-related changes in these variables (12, 13, 16, 20, 24, 46). Inspection of Figs. 1 and 2 provides a potential explanation for this finding: there are marked intersubject and intersample variations in these variables over the range of gestation studied. These variations likely reflect the continuous fluctuation in fetal vascular PO₂, PCO₂, pH, and O₂ saturation that occur in the fetal lamb in association with fetal breathing movements and uterine prelabor contractions (27, 33, 43, 57, 65, 71). Woudstra et al. (71) monitored blood gas and lactate concentrations in singleton, twins, and triplet fetal lambs.

Table 2. Blood gas and acid-base status and blood glucose and lactate concentrations in singleton, twins, and triplet fetal lambs

<table>
<thead>
<tr>
<th>Variable</th>
<th>Singletons (156)*</th>
<th>Twins (251)*</th>
<th>Triplets (35)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean GA, day</td>
<td>129.1 ± 0.4</td>
<td>126.9 ± 0.5</td>
<td>122.2 ± 1.5</td>
</tr>
<tr>
<td>PO₂, mmHg</td>
<td>23.7 ± 0.3</td>
<td>24.3 ± 0.3</td>
<td>21.2 ± 1.2</td>
</tr>
<tr>
<td>PCO₂, mmHg</td>
<td>48.5 ± 0.3</td>
<td>47.0 ± 0.3</td>
<td>47.6 ± 1.0</td>
</tr>
<tr>
<td>pH</td>
<td>7.331 ± 0.004</td>
<td>7.359 ± 0.003</td>
<td>7.359 ± 0.011</td>
</tr>
<tr>
<td>HCO₃⁻, meq/l</td>
<td>25.3 ± 0.2</td>
<td>25.8 ± 0.2</td>
<td>26.3 ± 0.4</td>
</tr>
<tr>
<td>TCO₂, meq/l</td>
<td>26.7 ± 0.2</td>
<td>27.2 ± 0.2</td>
<td>27.3 ± 0.4</td>
</tr>
<tr>
<td>BE, meq/l</td>
<td>0.4 ± 0.2</td>
<td>0.4 ± 0.2</td>
<td>2.0 ± 0.4</td>
</tr>
<tr>
<td>SO₂, %</td>
<td>52.0 ± 1.2</td>
<td>59.4 ± 1.0</td>
<td>50.6 ± 3.6</td>
</tr>
<tr>
<td>Hb, mg %</td>
<td>10.4 ± 0.1</td>
<td>10.5 ± 0.1</td>
<td>11.1 ± 0.3</td>
</tr>
<tr>
<td>CO₂, mM</td>
<td>3.3 ± 0.1</td>
<td>3.8 ± 0.1</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>[Glucose], mM</td>
<td>1.21 ± 0.04</td>
<td>0.70 ± 0.02</td>
<td>0.47 ± 0.03</td>
</tr>
<tr>
<td>[Lactate], mM</td>
<td>0.85 ± 0.02</td>
<td>1.20 ± 0.04</td>
<td>2.18 ± 0.06</td>
</tr>
</tbody>
</table>

All values are given as means ± SE. *Number in parentheses give the number of samples. a,b,cDifferent letter subscripts indicate statistically significant differences.
acid-base variables continuously for 25–28 h in five fetal lambs at ∼132 days of gestation. The minimum and maximum values for arterial Po2 (18.2 ± 1.5, 22.3 ± 1.6 mmHg), PCO2 (48.6 ± 1.2, 56.1 ± 1.7 mmHg), pH (7.346 ± 0.013, 7.413 ± 0.129), and SO2 (34.1 ± 1.2, 62.0 ± 2.5%) are nearly as great as the changes in these variables between 100 days gestation and term (see Table 1). When somatic activity is temporarily abolished in fetal lambs by neuromuscular blockade combined with inhibition of uterine prelabor contractions by maternal administration of a β-adrenergic agonist, the fluctuations in arterial Po2 and SO2 were virtually abolished (27). Thus, measurement of these variables in small numbers of fetuses and/or over a narrow range of gestational ages is unlikely to detect and gestational age-related changes.

In the human fetus, umbilical arterial and venous Po2 and maternal vascular Po2 in the intervillous space begin to decline as early as 16 wk of gestation (0.4 of term) (62). In the present study, the earliest samples were collected at 103 days (0.7 of term). In fetal lambs at 75 days of gestation (0.5 of term), arterial O2 saturation averaged 67.9 ± 1.5% (7). This is lower than the values measured at 103 days of gestation and calculated for 100 days (Fig. 2 and Table 2). Taken together, the current results and those of Bell et al. (7) suggest a pattern of gestational age-related changes in arterial O2 saturation similar to that reported from acute fetal lamb studies (3, 4, 35), with an increase between ∼60 and 100 days, then a decrease thereafter. Although this pattern should be verified with further studies, it suggests that it is different from that reported in the human fetus (62).

The mechanisms underlying the alterations in fetal blood gas status with advancing gestation very likely involve the progressive decrease in fetal weight-normalized uterine and umbilical blood flows as gestation proceeds (23, 30, 37, 42, 60). Although the absolute rates of uterine and umbilical blood flow increase progressively until at least late gestation, these rises do not keep pace with fetal growth, so that the weight-normalized rates fall. Such changes have been observed in a number of species, including human, sheep, horse, rabbit, and guinea pig (23, 37, 60). In pregnant sheep, acute experimental reductions in uterine or umbilical blood flow decrease fetal arterial oxygenation and pH and increase PCO2 (31, 34, 69, 70). These results suggest that reductions in flow in either vessel of ∼50% would be required to elicit the reduction in fetal arterial O2 saturation and content that occurs between ∼100 days gestation and term (see Table 1). The studies of gestational age-related changes in uterine and umbilical blood flows in sheep suggest that between 100 days gestation and term, the fetal weight-normalized value falls by ∼25% in each vessel (60). Although the combined effects of simultaneous experimental reductions in uterine and umbilical blood flows do not appear to have been studied, it would seem that 25% reductions in both weight-normalized uterine and umbilical blood flows are sufficient to result in the changes in gestational age-related fetal blood gas and acid-base status observed in the present study. What is less clear is why the increase in absolute uterine and umbilical blood flows cannot keep pace with fetal growth in late gestation, but it may be related to an upper limit of the proportions of cardiac output that the mother and fetus can divert to the placenta because of the need to maintain an adequate blood flow to other organs and tissues.

**Fetal lactate concentrations.** Although fetal arterial lactate concentration was not related to gestational age, it was inversely related to arterial Po2 and O2 content. Experimental hypoxemia in fetal lambs, even of a modest nature, results in an elevation in fetal plasma lactate concentration (64). Koos et al. (39) subjected fetal lambs at ∼130 days of gestation to graded hypoxemia and in a related article (38) estimated brain Po2 from the Po2 values obtained in samples of fetal carotid arterial and jugular venous blood. Severe hypoxemia resulted in a mean arterial Po2 of 16.8 ± 1.4 mmHg. Predicted mean brain tissue Po2 in this situation was 4.2 mmHg with −6% of the

Table 3. y-Intercept values and the regression coefficients for gestational age, fetal number, and fetal sex obtained from multiple regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Intercept</th>
<th>GA</th>
<th>Fetal Number</th>
<th>Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Po2</td>
<td>50.2 ± 4.7*</td>
<td>0.194 ± 0.035*</td>
<td>−0.940 ± 0.436*</td>
<td>−0.211 ± 0.522</td>
</tr>
<tr>
<td>PCO2</td>
<td>28.4 ± 4.1*</td>
<td>0.157 ± 0.031*</td>
<td>−0.518 ± 0.381</td>
<td>0.258 ± 0.456</td>
</tr>
<tr>
<td>pH</td>
<td>7.508 ± 0.044*</td>
<td>−0.0015 ± 0.004*</td>
<td>0.0157 ± 0.004*</td>
<td>0.0011 ± 0.0048</td>
</tr>
<tr>
<td>HCO3</td>
<td>26.04 ± 2.35*</td>
<td>−0.0009 ± 0.0175</td>
<td>0.49 ± 0.022</td>
<td>0.022 ± 0.261</td>
</tr>
<tr>
<td>TC02</td>
<td>27.68 ± 2.39*</td>
<td>−0.00103 ± 0.0178</td>
<td>0.364 ± 0.022</td>
<td>0.031 ± 0.266</td>
</tr>
<tr>
<td>BE</td>
<td>4.45 ± 2.46*</td>
<td>−0.037 ± 0.018*</td>
<td>0.766 ± 0.228*</td>
<td>0.136 ± 0.273</td>
</tr>
<tr>
<td>SO2</td>
<td>184.8 ± 13.2*</td>
<td>−1.01 ± 0.098*</td>
<td>0.354 ± 1.219</td>
<td>−1.537 ± 1.46</td>
</tr>
<tr>
<td>Hb</td>
<td>1.23 ± 1.41</td>
<td>0.067 ± 0.011*</td>
<td>0.52 ± 0.13*</td>
<td>−0.37 ± 0.16*</td>
</tr>
<tr>
<td>CO2</td>
<td>8.60 ± 0.86*</td>
<td>−0.0406 ± 0.006*</td>
<td>0.152 ± 0.080</td>
<td>−0.189 ± 0.096</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.118 ± 0.239*</td>
<td>−0.0006 ± 0.002</td>
<td>−0.181 ± 0.021*</td>
<td>0.239 ± 0.088*</td>
</tr>
<tr>
<td>Lactate</td>
<td>−1.23 ± 10.3</td>
<td>0.0162 ± 0.007*</td>
<td>0.135 ± 0.104</td>
<td></td>
</tr>
</tbody>
</table>

All values are given as means ± SE. GA, gestational age. *Significantly different from 0.

Fig. 5. The relationship between fetal arterial blood glucose concentration and birth weight in singleton (○), twin (●) and triplet (□) lambs born at term.
values being less than 1 mmHg (38). In the present study, the arterial Po2 below which plasma lactate levels rose was \( \sim 13 \) mmHg. As this value is lower than that seen with severe hypoxemia by Koos et al. (61), the results suggest that at a Po2 less than \( \sim 13 \) mmHg, tissue hypoxia could occur as a result of an inadequate O2 diffusion gradient between blood and the mitochondrial electron transport chain, leading to increased lactate production via anaerobic glycolysis. However, the source of the lactate is unclear. Under normal circumstances, lactate is produced by the placenta and released into the fetal circulation (61); thus, increased placental lactate production could contribute to the fetal lactic acidemia with hypoxemia. However, Limesand et al. (41) measured umbilical lactate uptake in fetal lambs rendered growth-restricted by maternal hyperthermia in early gestation. The mean fetal arterial Po2 was 11.7 mmHg, and the mean arterial lactate concentration was 3.98 mM. Using the regression equation from Fig. 4, we find that the lactate concentration would be 3.78 mM, which is close to the measured value. In the growth-restricted fetuses, lactate uptake from the placenta was significantly lower than in the control group (41). This suggests that with this degree of hypoxemia, the increased plasma lactate concentration is coming from fetal sources. From the studies of Koos and colleagues (38, 39), this could include the fetal brain. However, given the increase in fetal cerebral perfusion that occurs with hypoxemia (38), increased lactate production from other tissues in which perfusion is not increased, such as the carcass, seems more likely.

Blood gas and acid-base status in singletons, twins, and triplets. As noted in Table 2, the main differences between singleton, twin, and triplet lambs were with arterial Po2 and glucose and lactate levels. Compared with the singleton and twin Po2 values, the value in the triplets was slightly but significantly lower, whereas the lactate concentration was significantly higher. Arterial glucose concentration fell progressively from singletons to triplets. Multiple linear regression analysis confirmed these effects of fetal number and surprisingly demonstrated that increasing fetal number was associated with increased values for pH, HCO3-, and base excess.

An explanation for these findings is currently lacking. In terms of the effect of fetal number on arterial Po2, Christenson et al. (10) reported utero-ovarian blood flow in pregnant ewes at 105 and \( \sim 122 \) days gestation in singleton, twin, and triplet pregnancies. When the flow values were normalized to fetal weight, the values were not different. However, whether the decrease in weight-normalized uterine blood flow with advancing gestation that has been reported in sheep with singleton pregnancies (30, 60) occurs at a greater rate with twin or triplet pregnancies is not known. There also appear to be no measurements of umbilical blood flow in twin or triplet sheep pregnancies. However, cotyledonary and caruncular weight per fetus and capillary volumes decrease with increasing fetal number (66). These changes could affect the efficiency of placental O2 transfer, particularly over the course of gestation.

Given that arterial Po2 in twin fetuses was not different from that in singletons and that the value in triplets was only 2–3 mm lower than the values in singletons and twins, it seems unlikely that these differences in oxygen tension could be responsible for the decrease in birth weight with increasing fetal number. The view is supported by the fact that there was no relationship between fetal arterial Po2 and birth weight.

An explanation for the effect of increasing fetal number on arterial pH, HCO3-, and base excess is currently lacking. As all of these variables are related to blood CO2 concentration, the mechanism may involve fetal to maternal transfer of CO2. Although there were no significant differences in arterial Po2 in relation to fetal number, there was a trend for lower values in twins and triplets (Table 2), and this was also seen with multiple linear regression analysis, although again not significant (Table 3). In sheep, CO2 crosses the placenta primarily in the molecular form and not as HCO3- (44). However, in the human placenta, there is evidence for the presence a Cl/HCO3 exchanger (25, 40), and in the guinea pig, evidence for placental transfer of the HCO3- ion (28). Further research in this area is required before the reason for the increased pH and HCO3- with increasing fetal number can be elucidated.

In terms of the decrease in glucose concentration with increasing fetal number, Edwards and McMillen (18) have reported lower plasma glucose concentrations in twin compared with singleton fetal lambs, but this was not found by Vonnahme et al. (67). The mean blood glucose concentration in the triplets is similar to the values in studies in which experimental fetal growth restriction was achieved via surgical reduction of endometrial implantation sites (49) and maternal hyperthermia in early pregnancy (41). In the latter situation, umbilical glucose uptake was lower in the growth-restricted fetuses, whereas fetal glucose utilization rate was not different in the two groups. This was because of significant glucose production in the growth-restricted group, which was associated with increased hepatic mRNA concentrations of key gluconeogenic enzymes (41). Whether these changes in glucose metabolism also occur in twin and triplet lambs remains to be determined.

Fetal plasma glucose concentration falls between 76 and 132 days, and this leads to an increase in the maternal-fetal plasma glucose concentration gradient, thereby promoting the increase in placental glucose transfer capacity that occurs with advancing gestation (47). In singleton human fetuses, umbilical venous glucose concentration decreases between 16 and 42 wk, and the maternal-arterial-umbilical venous glucose concentration difference increases (45). The maternal-fetal glucose concentration difference was also increased in growth-restricted fetuses (45). There was no gestational age-related decrease in fetal arterial blood glucose concentration in the present study. However, in the study of Molina et al. (47), the earliest gestational age examined was 78 days, which was less than the earliest gestational age in the present study (103 days). Moreover, the rate of decline in glucose concentration may have been too low to detect in the present study. In addition, it is unclear whether the lower fetal arterial glucose concentration in twins and triplets would lead to increased umbilical glucose uptake, as Edwards and McMillen (18) reported that maternal plasma glucose concentration was lower in ewes carrying twins compared with those carrying singletons, and the maternal-fetal glucose concentration gradient was actually slightly lower in the twin-carrying ewes.

Birth weight in the triplet lambs (2.96 ± 0.23 kg) appears similar to the fetal weight in studies of experimental fetal growth restriction resulting from maternal hyperthermia in early pregnancy (8, 21) and carunclectomy (1, 54), although in some of these studies, fetal weight was determined before term. As noted above, blood glucose concentration in the triplets was...
similar to that in the growth-restricted fetuses. However, in at least one other respect, the triplet lambs are different from the growth-restricted lambs. Arterial Po2 in the triplets (21.2 ± 1.2 mmHg) is much higher than in the growth-restricted lambs in heat-stressed pregnancies [11.7 ± 1.2 mmHg (41)] and carunclecomized ewes [14.1 ± 0.1 mmHg (15)]. Prolonged maternal hypobaric hypoxia can result in reduced birth weight in sheep (32), and this has also been reported in some but not all studies of high-altitude hypoxia (51, 52). However, the results of the present study, showing no relationship between arterial Po2 and birth weight, and a significant positive relationship between glucose concentration and birth weight, and a significant positive relationship between arterial Po2 and birth weight, and a significant positive relationship between glucose concentration and birth weight (Fig. 5) suggests that with twin and particularly triplet fetal lambs, it is the hypoglycemia and perhaps reduced concentrations of other metabolic substrates (e.g., amino acids) and not hypoxemia that is primarily involved in the reduced birth weight. Further studies of fetal nutrient homeostasis in relationship to fetal number are required to substantiate this hypothesis.

Sex differences in fetal blood gas and acid-base status. The only sex difference in the analyzed data was with fetal hemoglobin concentration, which was significantly higher in females, a finding that does not appear to have been previously reported. Polglase et al. (53) have recently reported no sex differences in blood gas and acid-base status, including hemoglobin concentration, in term fetal lambs sampled immediately following cesarean delivery and artificial ventilation (n = 13 males, 9 females). However, in human infants and children up to 9 mo of age, hemoglobin concentration, mean corpuscular volume, and ferritin level are higher in females than males, and it was suggested that this was due to the sex differences in metabolism (17, 19). The higher hemoglobin concentration in human females appears to persist until about 11 yr of age, after which the concentration is higher in males (29). The reasons for the differences between the present study, the human postnatal studies, and the findings or Polglase et al. (53) are unclear but may relate to the larger sample size in the former studies. In addition, cesarean delivery of the lambs and subsequent ventilation may have altered hemoglobin concentration in the Polglase et al. study (53).

Conclusions. The results of this study indicate that from ~100 days gestation to term in fetal lambs, there is an alteration in arterial blood gas and acid-base status, comprising progressive decreases in Po2, pH, O2 saturation, and O2 content and a progressive increase in PCO2. These results are similar to those reported in the human fetus and are likely due to the decreases in weight-normalized uterine and umbilical blood flows that occur in both species. Increasing fetal number is associated with a lower Po2 and glucose concentration and higher pH, HCO3−, base excess, and lactate level. Overall, there was a significant relationship between glucose concentration and birth weight. This suggests that the reduced growth in twins and triplets is due more to reduced supply of glucose and perhaps other metabolic substrates (e.g., amino acids) rather than to hypoxemia.

Fetal weight-normalized uterine and umbilical blood flows decrease in human, sheep and several other species with advancing gestation (23, 30, 37, 60). The decrease in umbilical blood flow, coupled with the decrease in umbilical venous oxygenation, would decrease fetal oxygen delivery, and this has been reported in human pregnancy between 33 and 40 wk gestation (42). Fetal oxygen delivery normalized to fetal weight in sheep also likely falls with advancing gestation, given the higher rates of umbilical blood flow earlier in gestation (7). Moreover, fetal oxygen consumption normalized to wet or dry fetal weight is higher in lambs at midgestation compared with those at 119 and 141 days gestation (6). We have recently reported that body movements in fetal lambs decrease progressively with advancing gestation, beginning at 91.9 ± 5.2 days gestation, and this was followed by a decrease in the rate of increase in fetal abdominal diameter, which began at 113.1 ± 3.9 days (55). This suggests that a decrease in motility and growth may contribute to the reduction in fetal oxygen demands with advancing gestation. In addition, the reduced growth rate may involve a reduction in protein synthesis (36). Thus, the available data suggest that with advancing gestation, there are coordinated fetal responses to maintain the balance between oxygen delivery and consumption.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: D.R. conception and design of research; D.R. performed experiments; D.R. interpreted results of experiments; D.R. prepared figures; D.R. drafted manuscript; D.R. and N.W.B. approved final version of manuscript; N.W.B. analyzed data; N.W.B. edited and revised manuscript.

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