Ventilatory and arousal responses of sleeping lambs to respiratory challenges: effect of prenatal maternal anemia

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Moss, Timothy J., and Richard Harding. Ventilatory and arousal responses of sleeping lambs to respiratory challenges: effect of prenatal maternal anemia. J. Appl. Physiol. 88: 641–648, 2000.—We have examined the effects of exposure to chronic maternal anemia, throughout the final one-third of gestation, on postnatal ventilatory and arousal responses to hypoxia, hypercapnia, and combined hypoxia-hypercapnia in sleeping lambs. While resting quietly awake, lambs from anemic ewes had higher arterial PCO2 levels than control animals during the first 2–3 postnatal wk, but pH, arterial PO2, and arterial O2 saturation were not different. During active and quiet sleep lambs from anemic ewes had higher end-tidal CO2 levels than control animals when breathing room air and at the time of spontaneous arousal or when aroused by progressive hypercapnia or by combined hypoxia-hypercapnia. Ventilation and arterial O2 saturation during uninterrupted sleep and ventilatory responsiveness to hypoxia (inspiratory O2 fraction, 10%), progressive hypercapnia, and combined hypoxia/hypercapnia were not significantly affected by exposure to maternal anemia. Our findings show that maternal anemia results in elevated Pco2 levels in the offspring. This effect may be due, at least in part, to altered pulmonary function.

hypoxia; hypercapnia; low birth weight; intrauterine growth retardation

ACCUMULATING EVIDENCE SUGGESTS that many victims of sudden infant death syndrome have experienced prenatal compromise, resulting in intrauterine growth restriction (16, 21). However, few investigations of ventilatory control in newborns after exposure to adverse intrauterine conditions have been conducted.

We have recently shown that low-birth-weight lambs, unlike normal lambs, do not increase their ventilatory responsiveness to progressive hypoxia during wakefulness as they age (18). Because sudden infant death syndrome is normally associated with sleep (31), we thought it important to extend these observations and examine ventilatory responses to respiratory stimuli during sleep in lambs that had experienced prenatal compromise.

We chose to induce chronic anemia in pregnant ewes as a means of causing prenatal compromise in fetuses that could be subsequently studied after birth. Maternal anemia during human pregnancy is associated with increased risks of prematurity and intrauterine growth restriction (1). Chronic maternal anemia in a sheep model results in fetal growth restriction (20). It was our aim to determine whether ventilatory and arousal responses of newborn lambs to respiratory stimuli experienced during sleep are affected by prenatal exposure to chronic maternal anemia.

METHODS

Seven control lambs and six lambs from chronically anemic ewes were studied repeatedly during the first 2–3 postnatal wk. Six date-mated sheep underwent aseptic surgery (halothane anesthesia) at 91 ± 1 days postmating for the implantation of femoral artery and vein catheters to allow the induction, maintenance, and monitoring of chronic anemia. After surgery, arterial blood was sampled daily for measurement of arterial pH, PaCO2, PaO2 (Paco2, PaO2), and O2 saturation (SaO2; ABL510, Radiometer). Hematocrit was measured in triplicate by using microcapillary tubes. Beginning on day 96 of pregnancy, “exchange transfusions” (during which 500 ml of blood were replaced with plasma) were commenced in six ewes to lower the maternal hematocrit to below 14% (20). On day 143 ± 1 of pregnancy, catheters were cut short and obstructed, and ewes were allowed to spontaneously deliver lambs at term. Control lambs were obtained from ewes that did not experience any intervention during pregnancy.

After delivery, lambs were weighed and body dimensions and ponderal index [body wt/(crown-rump length)3] were measured. Postnatal lambs (1–3 days of age) underwent aseptic surgery (halothane anesthesia) for the implantation of a femoral artery catheter (for blood sampling), an intrapleural balloon-tipped catheter, and pairs of stainless steel electrodes (AS632, Cooner) to monitor electrocorticogram (ECoG), electrooculogram (EOG), and nuchal electromyogram (EMG). Signals from these electrodes were used in the identification of sleep states and arousal.

Studies of ventilation during sleep were performed daily beginning on the day after surgery until 13 ± 2 days of age; after this point, lambs were unlikely to sleep spontaneously in the laboratory. During studies, lambs were placed prone in a sling within a soundproof room. Rectal temperature was continuously monitored. While lambs rested quietly awake, an arterial blood sample was collected for determination of pH, PaCO2, PaO2, and SaO2 (ABL510, Radiometer). A pulse oximeter (N-200, Nellcor) was attached to the lamb's shaved tail to continuously measure transcutaneous O2 saturation (So2).

Lambs were fitted with a flexible face mask (Kruus) to allow measurement of ventilation. Before the face mask was fitted, the snout was shaved and dental impression material (President, Coltène, Switzerland) was applied to the inside rim of the mask to ensure an airtight seal. The mask was

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easily and painlessly removed at the end of each experiment. Gas was continually drawn from the face mask to record the inspired O₂ (F IO₂) and end-tidal CO₂ fractions (FETCO₂; Eliza Duo, Engström, Sweden). A pneumotachometer (model 4500A, Hans Rudolph) was inserted into the outlet of the face mask to measure ventilation; it was attached to a differential pressure transducer (model PT5A, Grass Instruments), and the amplified signal was integrated (model PT10, Grass Instruments) to yield a measurement of volume.

Physiological data were sampled at 200 Hz by an analog-to-digital converter (MacLab, AD Instruments) and recorded on a computer (PowerMac 7200, Apple). “Chart” software (AD Instruments) was used to record the integrated pneumotachometer signal and to calculate the volume of each breath (tidal volume; VT). Breathing frequency (f) was determined from the integrated pneumotachometer record. Electrode signals were conditioned and amplified (Grass Instruments) before being sampled.

During each study, one of three different ventilatory tests was performed (hypcapnia, combined hypoxia-hypcapnia, or hypoxia), or lambs were allowed to sleep without interruption. Manipulation of inspired gases was performed remotely from outside the soundproof room. When hypcapnic and combined hypoxic-hypcapnic tests were performed, a Y piece that allowed switching of airflow was attached to the pneumotachometer; one outlet of the Y piece was attached to a rubber bag, whereas the other port was open to room air. Between tests, lambs breathed room air via the open port. To perform hypcapnic and combined hypoxic-hypcapnic tests, the direction of airflow through the Y piece was switched so that the lamb breathed to and from the rubber bag. The bag contained 200 ml/kg body weight of gas consisting of either 5% CO₂-50% O₂-balance N₂ (for hypcapnic tests) or 5% CO₂-21% O₂-balance N₂ (for combined hypoxic-hypcapnic tests).

During studies when hypoxic tests were performed, the pneumotachometer was attached to the side port in a tube through which gas mixtures flowed. Between hypoxic tests, 21% O₂ in N₂ flowed through the tube at 5–6 l/min. To perform hypoxic tests, the O₂ content of the inspired gas was rapidly reduced to, and maintained at, 10%.

Ventilatory tests were performed during identified episodes of active sleep (AS; low-voltage ECoG, active EOG, inactive EMG) and quiet sleep (QS; high-voltage ECoG, inactive EOG, tonically active EMG). Ventilatory tests were discontinued when lambs aroused from sleep. Arousal from AS was defined as the appearance of nuchal EMG activity, and arousal from QS was defined as the switching from high- to low-voltage ECoG activity. Observation of lambs by using a video camera aided in the identification of sleep states and arousal. Up to seven ventilatory tests or episodes of uninterrupted sleep were recorded in each sleep state during each study.

Data analysis. Minute ventilation (VE; ml ·min⁻¹·kg⁻¹; the product of VT and f), FETCO₂, and SO₂ during uninterrupted sleep were determined from averages of VT, f, FETCO₂, and SO₂ measured during entire episodes of uninterrupted AS and QS.

To quantify ventilatory responses to hypercapnia and combined hypoxia-hypcapnia, each of the tests was divided into 5-s segments and mean VE and FETCO₂ values for each of these segments were determined. We calculated the gradient of the relationship between VE and FETCO₂ (from linear regression analysis; Fig. 1) to provide an index of ventilatory responsiveness (to hypercapnia or combined hypoxia/hypcapnia). Regression lines were also extrapolated to determine the FETCO₂ level at which ventilation equaled zero. We used this measurement as an index of the FETCO₂ level around which ventilatory responses were set.

To quantify ventilatory responsiveness to hypoxia, VE and SO₂ were averaged over 5 s, beginning 1 min after FIO₂ was reduced to 10%. As an index of hypoxic ventilatory responsiveness, we calculated the percent increase in ventilation above that measured immediately before the hypoxic test. To normalize ventilatory responsiveness, increases in ventilation in response to hypoxia were divided by the reduction in SO₂ that occurred during the test.

VE, VT, f, FETCO₂, SO₂, and the inspiratory deflections in intrapleural pressure were measured from the three breaths immediately preceding arousal to provide indexes of ventilatory effort and the degree of hypoxia or hypcapnia experienced during normal sleep and when ventilatory tests were performed. The duration of each sleep episode was also measured.

Statistical analyses. Statistical analyses were performed by using SAS software (SAS). Initial analysis indicated that no variables changed with postnatal age (2–17 days) over the study period. Therefore, resting ventilation, arousal, and ventilatory responsiveness measurements and measurements of PaO₂, PaCO₂, and pH were averaged for each day of study for each animal. Values were averaged to provide a single value for each animal.

Birth weights, crown-rump lengths, ponderal indexes, and measurements of rectal temperature, PaCO₂, PaO₂, and pH were compared between groups by using unpaired t-tests. Responses to ventilatory tests and measurements made at arousal were compared by repeated-measures ANOVA with one between-groups factor (control vs. anemia) and one within-groups factor [either 1) AS vs. QS or 2) test type]. Post hoc comparisons were made by using the least significant difference test. Values of P < 0.05 were considered statistically significant, and only significant differences are reported unless otherwise indicated. All data are expressed as means ± SE.

RESULTS

Hematocrits of ewes that underwent exchange transfusions are shown in Fig. 2. Exchange transfusions initially were performed twice daily (over 3–5 days) until the ewe’s hematocrit fell below 14%. After this time it was necessary to perform exchange transfusions at least twice every 3 days to maintain hematocrit at this reduced level. Maternal hematocrit was reduced from 33.5 ± 3.2% (before exchange transfusions were commenced) to 13.7 ± 0.2% throughout the anemic period.

Control and treated lambs were born spontaneously at full term (control, 147 ± 1 days; treated, 147 ± 1 days). Birth weights of treated lambs (3.9 ± 0.4 kg) were lower than those of control animals (5.2 ± 0.2 kg; Fig. 3). Treated lambs had lower ponderal indexes (2.53 ± 0.16 × 10⁻⁵ kg/cm³) than did control animals (3.15 ± 0.18 × 10⁻⁵ kg/cm³), resulting in treated lambs appearing thin and “wasted.” Parity was not different between the two groups of lambs (control, 1.4 ± 0.2; treated, 1.2 ± 0.2). At all ages, treated lambs weighed less than control animals (Fig. 3). The mean daily growth rate for control lambs (290 ± 30 g/day) was not different from that of treated lambs (220 ± 30 g/day).

Arterial pH (control, 7.41 ± 0.01; treated, 7.40 ± 0.01), PaO₂ (control, 97.0 ± 2.1 Torr; treated, 94.3 ± 3.4
Torr), and SaO₂ (control, 96.5 ± 0.6%; treated, 96.1 ± 1.6%) when lambs rested quietly awake breathing room air were not different between control and treated lambs. Mean PaCO₂ of treated lambs (42.4 ± 0.7 Torr) was greater than in control animals (39.9 ± 0.6 Torr). PaCO₂, pH, PaO₂, and SaO₂ did not change with age in either group. Rectal temperatures of control lambs (39.8 ± 0.01°C) were slightly higher than those of treated lambs (39.6 ± 0.03°C).

Measurements during sleep. V̇E during uninterrupted sleep was not different between groups but was lower in both groups during AS than during QS (Fig. 4). VT and f were not different between control and treated lambs but were both greater during QS than during AS (data not shown). In both groups, FETCO₂ was higher during AS than during QS and was greater in treated than control lambs during both sleep states (Fig. 4). So₂ was not different between groups but was lower during AS than during QS for both groups (Fig. 4).

Responses to combined hypoxia-hypercapnia were measured in all lambs, but responses to hypercapnia were not measured in one treated lamb. Ventilatory responses to hypercapnia and combined hypoxia-hypercapnia were not significantly different between control and treated lambs (P = 0.23), although there was a tendency for responses to be lower in the treated group (Fig. 5). In both groups, ventilatory responses to hypercapnia and combined hypoxia-hypercapnia were greater during QS than during AS. During QS, ventilatory responses to combined hypoxia-hypercapnia were greater than responses to hypercapnia (Fig. 5). Responses to hypercapnia and combined hypoxia-hyper-
capnia consisted of increases in both $V_T$ and $f$ for both groups of lambs (Fig. 5). The value of $F_{\text{ETCO}_2}$ at which ventilation equals zero (by extrapolation of the regression equation) was not altered by sleep state and was not different between hypercapnic and combined hypoxic-hypercapnic tests. There were no significant differences in these estimates of CO$_2$ set point between control ($3.9 \pm 0.7\% F_{\text{ETCO}_2}$) and treated lambs ($2.15 \pm 0.52\% F_{\text{ETCO}_2}$).

Responses to steady-state hypoxia ($F_{\text{IO}_2}, 10\%$) were determined in six control lambs in AS and QS but could only be measured in four treated lambs during AS and in three during QS. Increases in $V_E$ were not different between groups. In both groups, ventilatory increases during hypoxia tended to be greater ($P = 0.09$) during QS than during AS. In both groups, decreases in $S_O_2$ in response to hypoxia were greater during AS (control, $16.7 \pm 2.5\%$; treated, $16.1 \pm 3.3\%$) than during QS (control, $11.4 \pm 2.4\%$; treated, $11.8 \pm 4.2\%$).

Fig. 2. Hematocrits of 6 ewes that underwent exchange transfusions (○) and a group of 6 unbled ewes (●). Arrow indicates point at which exchange transfusions commenced. Timing of birth of lambs from anemic ewes (means ± SE) is indicated.

Fig. 3. Body weights of lambs from anemic ewes (○) and control lambs (●) at birth and during first 2 postnatal wk. Values are means of individual measurements made on days when studies of ventilation during sleep were conducted.

Fig. 4. Minute ventilation, end-tidal CO$_2$, and O$_2$ saturation ($S_O_2$) averaged in control lambs (solid bars) and lambs from anemic ewes (open bars) throughout entire episodes of uninterrupted active and quiet sleep. All 3 variables were significantly different during quiet sleep than during active sleep for both groups of lambs. End-tidal CO$_2$ fraction ($F_{\text{ETCO}_2}$) was significantly higher in treated than in control lambs during both sleep states. Minute ventilation and $S_O_2$ were not different between control and treated lambs.

responses to hypoxia (increase in $V_E$/decrease in $S_O_2$) were not different between groups but were lower during AS than during QS (Fig. 5). Responses to hypoxia during AS and QS consisted of increases in $V_T$ and $f$ for both groups of lambs.

Fig. 4. Minute ventilation, end-tidal CO$_2$, and O$_2$ saturation ($S_O_2$) averaged in control lambs (solid bars) and lambs from anemic ewes (open bars) throughout entire episodes of uninterrupted active and quiet sleep. All 3 variables were significantly different during quiet sleep than during active sleep for both groups of lambs. End-tidal CO$_2$ fraction ($F_{\text{ETCO}_2}$) was significantly higher in treated than in control lambs during both sleep states. Minute ventilation and $S_O_2$ were not different between control and treated lambs.

Measurement of ventilatory responses to hypoxia (increase in $V_E$/decrease in $S_O_2$) were not different between groups but were lower during AS than during QS (Fig. 5). Responses to hypoxia during AS and QS consisted of increases in $V_T$ and $f$ for both groups of lambs.

Ventilation during AS and QS were not different between AS and QS for both groups of lambs and were not different between treatment groups for either sleep state. Similarly, lambs spent more time in AS than QS when ventilatory tests (hypercapnia, combined hypoxia-hypercapnia, or hypoxia) were performed. The duration of AS episodes was shortened by hypercapnic, combined hypoxic-hypercapnic, and hypoxic tests. QS episodes were shorter when hypercapnic and combined hypoxic-hypercapnic tests were performed but not when hypoxic tests were performed.

$V_E$ at arousal was not different between sleep states but was significantly greater when hypercapnic and combined hypoxic-hypercapnic (but not hypoxic) ventilatory tests were performed than at arousal from
uninterrupted sleep. Accordingly, inspiratory effort (i.e., inspiratory deflection in intrapleural pressure) was significantly greater immediately before arousal from hypercapnic and combined hypoxic-hypercapnic tests during QS than when no tests were performed in this state. In both groups, SO$_2$ at arousal from combined hypoxic-hypercapnic and hypoxic tests during AS, but not QS, was lower than when no ventilatory tests were performed. In both sleep states, F$_{ETCO2}$ at arousal was significantly greater in treated lambs than in control lambs when hypercapnic, combined hypoxic-hypercapnic, or no ventilatory tests were performed. In both groups, F$_{ETCO2}$ at arousal from AS was greater than at arousal from QS; this sleep-state-related difference was also present when hypercapnic and combined hypoxic-hypercapnic tests were performed. In both groups of lambs, F$_{ETCO2}$ was greater at arousal from hypercapnic tests than at arousal from combined hypoxic-hypercapnic tests for both sleep states.

**DISCUSSION**

Exposure to maternal anemia throughout late gestation resulted in restricted fetal growth and chronic elevations in CO$_2$ levels throughout early postnatal life. Despite this relative hypercapnia, arterial pH, P$_{A02}$, Sa$_{O2}$, and ventilation were not significantly affected during sleep or wakefulness. Ventilatory responses to hypercapnia and combined hypoxia-hypercapnia tended to be reduced in treated lambs, whereas responses to hypoxia appeared to be unaffected.

We have extended the findings of Mostello et al. (20) by demonstrating that fetuses from chronically anemic ewes can survive this prenatal insult and pregnancies proceed to full-term. We have shown that lambs from anemic ewes are growth restricted at birth; their birth weights are low, but measurements of body dimensions are not affected, resulting in a thin, wasted appearance. In contrast, human neonates born to anemic mothers...
Table 1. Measurements made at arousal from normal sleep and when combined hypoxic/hypercapnic, hypercapnic, and hypoxic ventilatory tests were performed in control and treated lambs

<table>
<thead>
<tr>
<th></th>
<th>Normal Sleep</th>
<th>Combined Hypoxia-Hypercapnia</th>
<th>Hypercapnia</th>
<th>Hypoxia</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Anemia</td>
<td>Control</td>
<td>Anemia</td>
</tr>
<tr>
<td>Duration of sleep, s</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AS</td>
<td>230.4±24.4</td>
<td>187.0±32.7</td>
<td>105.9±12.4</td>
<td>89.4±14.4</td>
</tr>
<tr>
<td>QS</td>
<td>[101.7±8.3]</td>
<td>[101.4±14.4]</td>
<td>[68.1±12.5]</td>
<td>[45.4±3.3]</td>
</tr>
<tr>
<td>VT, ml/mg/kg</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>AS</td>
<td>11.0±0.8</td>
<td>13.1±1.2</td>
<td>16.1±2.8</td>
<td>17.7±1.7</td>
</tr>
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<td>QS</td>
<td>10.4±0.7</td>
<td>12.1±0.8</td>
<td>18.1±1.9</td>
<td>17.8±2.5</td>
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<tr>
<td>f, breaths/ min</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>AS</td>
<td>53.6±3.3</td>
<td>50.6±4.0</td>
<td>73.2±3.4</td>
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<td>QS</td>
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<td>57.4±4.1</td>
<td>86.1±3.2</td>
<td>73.9±7.9</td>
</tr>
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<td>Ve, ml.min⁻¹.kg⁻¹</td>
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<td></td>
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<tr>
<td>AS</td>
<td>570.1±48.9</td>
<td>599.1±16.0</td>
<td>1,203.7±263.6</td>
<td>1,173.4±86.8</td>
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<td>590.1±44.8</td>
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<td>FETCO₂, %</td>
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<td>AS</td>
<td>5.4±0.2‡</td>
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<tr>
<td>QS</td>
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<td>[7.3±0.3†]</td>
<td>[8.1±0.1‡]</td>
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<td>SO₂, %</td>
<td>92.6±1.9</td>
<td>94.7±1.3</td>
<td>74.9±4.6</td>
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<td>IPP₁, mmHg</td>
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<td>97.3±0.8</td>
<td>[91.7±1.5]</td>
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<td>[15.9±2.0]</td>
<td>[11.9±3.8]</td>
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<tr>
<td>QS</td>
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<td>10.2±1.4</td>
<td>[16.4±2.2]</td>
<td>[9.5±3.2]</td>
</tr>
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</table>

Values are means ± SE. AS, active sleep; QS, quiet sleep; VT, tidal volume; Ve, minute ventilation; FETCO₂, end-tidal CO₂ fraction; SO₂, O₂ saturation; IPP₁, inspiratory inspiral air pressure. *Significantly different between the test condition and interrupted normal sleep (data from both treatment groups combined), P < 0.05. †Significant differences between sleep states (data from both treatments combined), P < 0.05. ‡Significant differences between control lambs (control) and lambs from anemic ewes (anemia), P < 0.05.

have shorter body lengths as well as low birth weights (27). The type of growth restriction that we observed is typical of that caused by intrauterine compromise during late gestation (15), whereas the overall retardation of growth observed in humans may reflect an effect of intrauterine compromise from earlier in gestation. Like the low-weight-lamb lambs that we have studied previously (18), lambs from anemic ewes grew at a rate similar to control lambs during the first few postnatal weeks, resulting in their body weights remaining below those of control animals throughout the study period. This observation is consistent with studies of postnatal growth in infants small for their gestational age who were born at or before full term (11, 29).

The intrauterine growth restriction we observed was presumably due to a reduced nutrient supply to fetuses of anemic ewes because chronic maternal anemia throughout the final one-third of gestation in this species has been shown to cause a failure of uterine artery blood flow to increase with gestational age (20). Despite this reduced uterine blood flow, fetuses from anemic ewes studied by Mostello et al. (20) were not chronically hypoglycemic or hypoxicemic, and hematocrit was not elevated. However, in our study, the presence of fetal hypoxemia and/or hypoglycemia cannot be excluded because the period of exposure to maternal anemia was ~2 wk greater than that of the fetuses studied by Mostello et al. Thus our lambs may have been more affected because of the increased duration of exposure to maternal anemia.

Mostello et al. (20) reported a tendency for FETCO₂ to be higher in fetuses from anemic ewes than in control animals (39.5 ± 0.5 vs. 36.2 ± 0.8 Torr, P = 0.08), and this intrauterine hypercapnia (which may have been greater in our fetuses owing to the longer exposure to maternal anemia) may have altered the CO₂ ventilatory set point during prenatal development. We have attempted to determine CO₂ set points by extrapolation of the linear regression relationship between ventilation and FETCO₂. These estimates were not different between control and treated lambs; however, they may be unreliable because we have not been able to demonstrate an effect of oxygenation on these estimates (4). Lambs from anemic ewes had higher FETCO₂ levels at spontaneous arousal from AS and QS. Similarly, FETCO₂ at arousal was higher in treated lambs when hypercapnic and combined hypoxic-hypercapnic ventilatory responses were performed, confirming a tolerance of these lambs for higher levels of CO₂. The elevated PCO₂ levels in lambs from anemic ewes could be due to impaired pulmonary gas exchange or airway function. We did not collect data relating to lung function in our lambs, but previous studies suggest that altered pulmonary development could be responsible for our observations. In humans, low birth weight is associated with impaired lung function in childhood (25) and adult life (2, 28), and this is believed to be due to constrained airway growth during gestation. Experimental growth restriction in utero, because of placental insufficiency, affects the development of the trachea in sheep, and it is possible that more distal airways are similarly affected (23). In addition, recent experiments performed in our laboratory have demonstrated abnormalities in pulmonary structure (thickened air-blood...
barrier) and function (increased chest wall and decreased lung compliance), persisting for at least 8 wk, in lambs that were growth restricted in utero; these lambs had elevated PaCO₂ and reduced PaO₂ levels (30). Thus it is possible that lambs from anemic ewes possess pulmonary abnormalities that restrict alveolar ventilation and hence elevate PaCO₂. Impaired gas diffusion across the pulmonary membrane seems an unlikely explanation for our present observations because CO₂ levels were affected without an effect on Po₂ or So₂. A further possible explanation for the elevated CO₂ levels observed in treated lambs is that CO₂ production may have been increased. However, we collected no data relating to O₂ consumption or CO₂ production and are unaware of studies specifically examining the effects of intrauterine growth restriction on these variables.

Lambs from anemic ewes did not have ventilatory responses to hypercapnia or combined hypoxia-hypercapnia that were significantly different from those of control lambs. There was, however, a tendency for ventilatory responsiveness to hypercapnia and combined hypoxia-hypercapnia to be lower in treated lambs than in controls. Variability of ventilatory responses was observed during AS and QS in both groups of lambs, and thus, although the mean ventilatory response of lambs from anemic ewes to hypercapnia appears low, values in these lambs were within the range observed in control lambs. Variability of ventilatory responses to hypercapnia has been demonstrated in lambs during wakefulness (19) and in human infants during sleep (5). Unlike human infants, who display greater variability in ventilatory responses during AS than during QS (5), in our lambs variability in responses appeared similar during AS and QS.

Although we found no significant differences in ventilatory responses to hypoxia, hypercapnia, or combined hypoxia-hypercapnia between lambs from anemic ewes and control lambs, responses of both groups were affected by sleep state, in concurrence with previous studies (5, 8, 10, 13, 24). This effect of sleep state was also present for arousal responses. When hypoxic, hypercapnic, and combined hypoxic-hypercapnic ventilatory tests were performed, arousal from AS was delayed when compared with QS. During hypercapnic and combined hypoxic-hypercapnic tests, greater degrees of hypercapnia were experienced during AS, and, when combined hypoxic-hypercapnic and hypoxic tests were performed in AS, greater degrees of hypoxia were experienced. These findings demonstrate that, in lambs, AS is associated with reduced ventilatory and arousal responsiveness and hence increased vulnerability to respiratory challenges.

As in previous studies of developing animals (17, 26, 33), we found a positive interaction between hypoxia and hypercapnia in stimulating ventilation. The absence of this interaction during AS may be due to the weak association between respiratory stimuli and ventilation in this state (22). A positive interaction between hypoxia and hypercapnia in stimulating arousal from QS and AS was also present in both groups of lambs.