Placental insufficiency improves when intrauterine growth-restricted fetal sheep are administered daily $\omega-3$ polyunsaturated fatty acid infusions

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INTRODUCTION

Intrauterine growth restriction (IUGR) is the failure of the fetus to reach its genetic growth potential due to poor intrauterine conditions. It occurs in both humans and livestock (Yates et al., 2018). Low birthweight due to IUGR is associated with high risk for perinatal morbidity and mortality as well as for metabolic health consequences. In humans, these manifest in obesity, diabetes, hypertension, and other metabolic conditions that diminish the quality of life (Yates et al., 2018). In livestock, they reduce growth efficiency and alter body composition (Gibbs et al., 2020). Maternal stress disrupts fetal nutrition during gestation by impairing placental development and thus limiting its capacity for delivering nutrients and O$_2$ to the fetus. Placental insufficiency impacts fetal programming, particularly in muscle, which results in offspring with decreased muscle growth and poor metabolic efficiency (Posont et al., 2021). We previously showed that maternofetal inflammation during late gestation leads to an IUGR phenotype near-term and after birth (Cadaret et al., 2019; Posont et al., 2021). Previous studies have explored the benefits of supplementing $\omega-3$ polyunsaturated fatty acids (PUFAs) on fetal outcomes due to their natural anti-inflammatory properties (Rosa Velazquez et al., 2020; Rosa-Velazquez et al., 2021), but only via maternal supplementation and only in uncompromised pregnancies. In this study, we hypothesized that targeting fetal and placental inflammation with daily $\omega-3$ PUFA infusion into the IUGR fetus would improve placental function. Thus, the objective of this study was to assess the impact of fetal eicosapentaenoic acid (EPA) administration on fetal nutrient and O$_2$ concentrations and their maternofetal gradients.

MATERIALS AND METHODS

These studies were approved by the Institutional Animal Care and Use Committee at the University of Nebraska-Lincoln, which is accredited by AAALAC International. Commercial Polypay ewes were timed-mated to a single sire, housed individually, and managed as described for previous studies (Cadaret et al., 2019; Posont et al., 2021). Using simple randomization, ewes were assigned to produce control or placental insufficiency-induced IUGR fetuses via the meticulously researched maternal hyperthermia model as previously described (Yates et al., 2019). Ewes producing control fetuses ($n = 12$) were pair-fed and housed at thermoneutral conditions of 25 °C. Ewes producing placental insufficiency-induced IUGR fetuses ($n = 8$) were kept at 40 °C + 35% relative humidity from the 40th to the 95th d of gestational age (dGA) and then at 25 °C thereafter.

At 118 dGA, indwelling Tygon catheters (US Plastics) were surgically placed in the fetal femoral artery and vein for blood sampling following our previous protocol (Cadaret et al., 2019). IUGR
fetuses were randomly assigned to receive daily IV infusions of 25 mg/kg/d EPA (Sigma-Aldrich) suspended in 10 mL of sterile saline (i.e., IUGR + EPA; n = 4) for 5 ± 1 d, beginning on dGA 121 ± 1. Untreated IUGR fetuses (n = 4) and controls (n = 12) received infusions of saline only. All infusion occurred over 1 h.

Simultaneous maternal and fetal blood samples were collected into heparinized syringes at −10, 45, 120, and 360 min from the initiation of each infusion. Maternal blood was collected via jugular venipuncture and fetal blood was collected via arterial catheters. The total blood volume sampled never exceeded 20 mL/d for ewes or 5 mL/d for fetuses. An ABL90 FLEX blood gas analyzer (Radiometer) was used to determine concentrations of glucose, lactate, Na⁺, K⁺, Cl⁻, and Ca++, as well as, partial pressures of O₂ (pO₂) and CO₂ (pCO₂) from maternal and fetal whole blood (Cadaret et al., 2019).

To determine the effects of the experimental group, time from initiating EPA infusion and their interaction, hematology and blood/oximetry variables were analyzed by ANOVA using the mixed procedure with repeated measures from SAS 9.4 (SAS Institute). Day of gestation was treated as a covariate. Each ewe/fetus combination was considered an experimental unit for all variables. Significant differences were identified by P-values of ≤ 0.05, and P-values of ≤ 0.10 were considered tendencies. All data are expressed as the mean ± standard error.

**RESULTS**

No experimental group × time interactions occurred for any of the presented variables. No differences were observed among experimental groups for any of the maternal blood values. Fetal blood glucose was less (P < 0.05) for IUGR compared with controls and greater (P < 0.05) for IUGR + EPA compared with controls, regardless of time from infusion (Table 1). Maternofetal gradient for glucose was greater (P < 0.05) for IUGR than for control or IUGR + EPA and was greater (P < 0.05) for IUGR + EPA than for controls (Figure 1A). Fetal pO₂ was less (P < 0.05) for IUGR compared with controls or IUGR + EPA and was less (P < 0.05) for IUGR + EPA than for controls. Maternofetal gradient for pO₂ was greater (P < 0.05) for IUGR than for controls but did not differ between IUGR + EPA and controls (Figure 1B). Fetal lactate was greater (P < 0.05) for IUGR compared with IUGR + EPA or controls and was greater (P < 0.05) for IUGR + EPA than for controls. Maternofetal gradient for lactate was greater (P < 0.05) for IUGR than for controls but did not differ between IUGR + EPA and controls (Figure 1C). Fetal pCO₂ was greater (P < 0.05) for IUGR than for controls but did not differ between IUGR + EPA + and controls (Figure 1D).

Fetal blood Na⁺ concentrations were greater (P < 0.05) for IUGR and IUGR + EPA than for controls. Maternofetal gradient for Na⁺ did not differ among groups (Figure 2A). Fetal blood K⁺ was greater (P < 0.05) for IUGR than for IUGR + EPA or controls and was greater (P < 0.05) for IUGR + EPA than for controls. Maternofetal gradient for K⁺ did not differ among groups (Figure 2B). Fetal blood Cl⁻ concentrations were greater (P < 0.05) for IUGR compared with control or IUGR + EPA and were greater (P < 0.05) for IUGR + EPA than for controls. Maternofetal gradient for Cl⁻ was less (P < 0.05) for IUGR than for controls but did not differ between IUGR + EPA and controls (Figure 2C). Fetal blood Ca++ concentrations were less (P < 0.05) for IUGR and IUGR + EPA than for controls. Maternofetal gradients for Ca++ did not differ among groups (Figure 2D).

**DISCUSSION**

In this study, we found that daily fetal ω-3 PUFA infusions during late gestation rescued several components of placental function that had been impaired by IUGR. As with our previous studies (Cadaret et al., 2019; Posont et al., 2021), IUGR fetuses in the present study were hypoglycemic. However, administration of the potent bioactive ω-3 PUFA, EPA, reduced the maternofetal gradient for glucose and returned IUGR fetuses to a euglycemic state, presumably by enhancing placental function over 1 h.

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**Table 1. Blood values for carbohydrates, gases, and electrolytes in IUGR fetal sheep**

| Fetal blood parameter | Control | IUGR | IUGR + EPA | P-value |
|-----------------------|---------|------|------------|---------|
| Glucose, mM           | 0.86 ± 0.02a | 0.76 ± 0.05b | 0.93 ± 0.02c | < 0.05  |
| pO₂, mmHg             | 16.4 ± 0.2a  | 12.9 ± 0.4b  | 15.6 ± 0.3c | < 0.05  |
| Lactate, mM           | 1.47 ± 0.04a | 2.26 ± 0.18b | 1.64 ± 0.05c | < 0.05  |
| pCO₂, mmHg            | 49.6 ± 0.2a  | 54.1 ± 0.9b  | 50.2 ± 0.3c | < 0.01  |
| Na⁺, mM               | 143.3 ± 1.1a | 152.5 ± 0.3b | 148.3 ± 0.6c | < 0.01  |
| K⁺, mM                | 3.73 ± 0.03a | 4.19 ± 0.06b | 3.94 ± 0.05c | < 0.05  |
| Cl⁻, mM               | 107.7 ± 0.4a | 112.2 ± 1.0b | 109.8 ± 0.6c | < 0.01  |
| Ca++, mM              | 1.22 ± 0.01a | 1.35 ± 0.01b | 1.36 ± 0.01b | < 0.05  |

a,b,c means with differing superscripts differ (P < 0.05).
transport of maternal glucose. Likewise, EPA administration reduced maternofetal O$_2$ and CO$_2$ gradients and recovered blood concentrations for both in IUGR fetuses, demonstrating an improvement in gas exchange across the IUGR placenta. Improving oxemia levels of the IUGR fetus via EPA infusions appeared to decrease glycolytic lactate production by fetal skeletal muscle, resulting in less circulating lactate. Interestingly, the benefits of fetal EPA infusion for electrolyte transport did not occur to the same degree as those observed for glucose and O$_2$. Indeed, maternofetal gradients for Na$^+$, K$^+$, and Ca$^{++}$ were not improved at all by EPA infusion, which might be attributable to the differing mechanism of placental transport and differing rates of placental utilization or storage among gases, carbohydrates, and electrolytes (Gaccioli and Lager, 2016). Although maternofetal homeostasis of electrolyte is less understood, it is likely that facilitated diffusion out of the placenta and into the umbilical occurs at a rate that is independent of placenta uptake, as the basal membranes both express transporters. Nevertheless, systemic fetal inflammation in late gestation has been shown to coincide with the diminished capacity of the placenta to meet the fetus’ nutritional needs, which results in the hallmark growth and metabolic pathologies of IUGR (Posont et al., 2018; Cadaret et al., 2019; Posont et al., 2021). From the findings of the present study, we can conclude that diminished placental transport in IUGR fetal sheep was improved with fetal ω-3 PUFA infusions, presumably due to their potent anti-inflammatory properties.

**IMPLICATIONS**

This study produced evidence that placental function was improved when IUGR fetuses were administered daily ω-3 PUFA infusions. These findings support our belief that fetal/placental inflammation plays a major role in placental insufficiency and that anti-inflammatory ω-3 PUFAs are a reasonable intervention for improving IUGR outcomes. Although intravenous infusion of fetuses may lack practicality for humans and livestock, it is worth
noting that fatty acids readily cross the placenta and thus should reach the fetus even when delivered maternal. Thus, further research is warranted to assess the effectiveness of maternal ω-3 PUFA supplementation in the intervention of IUGR.

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