Effects of Late-Gestation Fetal Intrauterine Growth Restriction on Extracellular Matrix Synthesis in Fetal Liver of Mongolian Sheep Postprint

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Abstract

This experiment aimed to investigate the effects of intrauterine growth restriction (IUGR) during late gestation on extracellular matrix synthesis in the fetal liver of Mongolian sheep. Eighteen healthy Mongolian sheep that had undergone estrus synchronization and conception were randomly divided into 3 groups with metabolizable energy levels of 0.175 (R1 group), 0.330 (R2 group), and 0.670 MJ/ (kg BW0.75 · d) (ad libitum feeding group, C group), respectively, with 6 replicates per group and 1 sheep per replicate. The ewes were slaughtered at 140 d of gestation to determine the number of parenchymal cells and extracellular matrix content in the fetal liver. The results showed that the fetal liver weight ($P<0.01$), hepatocyte number ($P<0.05$), hepatocyte nuclear diameter ($P<0.01$), and contents of type I collagen ($P<0.01$), type III collagen ($P<0.05$), type IV collagen ($P<0.01$), laminin ($P<0.01$), hyaluronic acid ($P<0.05$), and fibronectin ($P<0.01$) in the R1 group were significantly or extremely significantly lower than those in the C group, while the endothelial cell number was significantly higher than that in the C group ($P<0.05$); the fetal liver weight ($P<0.05$) and fibronectin content ($P<0.01$) in the R2 group were significantly or extremely significantly lower than those in the C group, while the endothelial cell number was significantly higher than C ($P<0.05$). The results suggest that nutritional restriction of Mongolian sheep during late gestation severely limits fetal liver growth and development; when the metabolizable energy level is 0.175 MJ/ (kg BW0.75 · d) , fetal liver extracellular matrix synthesis is severely affected and a hepatic fibrotic repair response occurs; when the metabolizable energy level is 0.330 MJ/ (kg BW0.75 • d) , only the fetal liver fibronectin content is altered.

Full Text

Effects of Fetal Intrauterine Growth Retardation during Late Pregnancy on Extracellular Matrix Synthesis in Fetal Liver of Mongolia Sheep

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Abstract

This study investigated the effects of fetal intrauterine growth retardation (IUGR) during late pregnancy on extracellular matrix synthesis in the fetal liver of Mongolia sheep. Eighteen healthy Mongolia sheep that had undergone synchronized estrus and conception were randomly allocated to three groups with six replicates per group (one sheep per replicate). The groups received metabolizable energy (ME) levels of 0.175 (R1 group), 0.330 (R2 group), and 0.670 MJ/(kg BW · ·d) (ad libitum group, C group), respectively. At day 140 of gestation, ewes were slaughtered to determine parenchymal cell numbers and extracellular matrix content in fetal livers. The results showed that fetal liver weight ($P<0.01$), hepatocyte number ($P<0.05$), hepatocyte nuclear diameter ($P<0.01$), and contents of collagen type I ($P<0.01$), collagen type III ($P<0.05$), collagen type IV ($P<0.01$), laminin ($P<0.01$), hyaluronic acid ($P<0.05$), and fibronectin ($P<0.01$) in the R1 group were significantly or extremely significantly lower than those in the C group, while endothelial cell number was significantly higher ($P<0.05$). The R2 group exhibited significantly lower fetal liver weight ($P<0.05$) and fibronectin content ($P<0.01$) compared to the C group, with significantly higher endothelial cell number ($P<0.05$). These findings indicate that severe nutritional restriction during late pregnancy in Mongolia sheep substantially limits fetal liver growth and development. At an ME level of 0.175 MJ/(kg BW · ·d), fetal liver extracellular matrix synthesis was severely impaired and hepatic fibrosis repair responses were triggered. At an ME level of 0.330 MJ/(kg BW · ·d), only fetal liver fibronectin content was altered.

Keywords: nutritional restriction; fetal liver; liver parenchymal cells; extracellular matrix

Introduction

Intrauterine growth retardation (IUGR), also known as fetal growth restriction (FGR), refers to a condition where embryonic body weight or organs fail to achieve their genetic growth potential, resulting in retarded fetal and organ growth and development. IUGR sheep fetuses inevitably exhibit congenital liver deficiencies that significantly affect postnatal liver growth and physiological function. As a functional organ, many liver functions are influenced by extracellular matrix (ECM) synthesis. ECM plays crucial roles in biological tissue construction, cell polarity maintenance, embryonic development, and cell differentiation. The main components of ECM include collagen types I, III, and IV, hyaluronic acid, laminin, and fibronectin. Liver injury leads to decreased ECM secretion, and disordered ECM metabolism forms the basis of hepatic fibrosis. Changes in ECM secretion often cause complications resulting in perinatal or early infant mortality. Given ECM' s important role in all stages of liver disease, investigating the effects of late-pregnancy IUGR on fetal liver ECM synthesis is beneficial for early liver disease prevention and improving survival rates. This study aimed to examine the impact of IUGR during late pregnancy on ECM synthesis in Mongolia sheep fetal livers, providing a theoretical basis for research on maintaining the balance between ECM synthesis and degradation during gestation and for scientific feeding management of pregnant ewes.

Materials and Methods

1.1 Experimental Animals and Feeding

Eighteen healthy Mongolia sheep of moderate body condition and 2-3 parity that had undergone synchronized estrus and conception (confirmed as singleton pregnancies via B-ultrasound analysis using a Medison SA-600 instrument) were randomly divided into three groups according to body weight. The groups received metabolizable energy levels of 0.175 (restriction group 1, R1), 0.330 (restriction group 2, R2), and 0.670 MJ/(kg BW ・ ・d) (ad libitum group, C), with six replicates per group (one sheep per replicate). Custom-made artificial bags were placed over the muzzles of restricted group ewes to provide alfalfa hay. After a one-week adaptation period, feed was supplied according to the designated amounts, while the C group received ad libitum access. All experimental sheep were fed twice daily at 08:30 and 16:00; the C group received an additional feeding at 11:00 to ensure adequate intake. All groups had free access to water and mineral salt blocks. Daily samples of consumed forage and residual forage were collected, mixed, and used for nutritional level determination. Forage and nutrient intake levels, as well as nutritional levels of fed and residual forage, are presented in .

1.2 Slaughter Method

At day 140 of gestation, six ewes from each group were slaughtered after 24 hours of feed deprivation and 15 hours of water deprivation, and live weight

before slaughter was recorded. After skinning, the abdomen was opened along the midline, fetuses were removed, and fetal livers were immediately extracted intact, weighed, and stored at -80°C for subsequent analysis.

1.3 Determination of Parenchymal Cell Numbers in Fetal Liver

The liver was incised at the hepatic hilum, and a 1 cm × 1 cm × 1 cm tissue sample was taken from the largest cross-section to prepare paraffin blocks. Sections were prepared using a microtome and stained with hematoxylin-eosin (HE). Hepatocyte number, hepatocyte nuclear diameter, endothelial cell number, and Kupffer cell number were measured using the professional image analysis software Image-pro Plus 6.

1.4 Determination of ECM in Fetal Liver

Approximately 0.5 g of fetal liver tissue from each group was homogenized in 9 volumes of 0.85% cold physiological saline. After centrifugation at 3,000 r/min for 15 minutes, the supernatant was collected. Enzyme-linked immunosorbent assay kits (Nanjing Jiancheng Bioengineering Institute) were used to determine contents of collagen type I (H142), collagen type III (H144), collagen type IV (H145), laminin (H148), and hyaluronic acid (H141) in fetal livers, with all measurements performed strictly according to manufacturer protocols.

1.5 Data Processing

All data were analyzed using the general linear model in SAS 9.0, with Duncan' s multiple range test used for post-hoc comparisons.

Results

2.1 Effects of IUGR during Late Pregnancy on Fetal Liver Weight in Mongolia Sheep

The effects of IUGR during late pregnancy on fetal liver weight are shown in . At day 140 of gestation, fetal body weight and liver weight in the R1 group were extremely significantly lower than those in the C group ($P<0.01$), with no significant difference in liver weight/body weight ratio between the two groups ($P>0.05$). The R2 group exhibited significantly or extremely significantly lower fetal body weight ($P<0.01$) and liver weight ($P<0.05$) compared to the C group, with no significant difference in liver weight/body weight ratio ($P>0.05$).

2.2 Effects of IUGR during Late Pregnancy on Parenchymal Cell Numbers in Fetal Liver

The effects of IUGR during late pregnancy on parenchymal cell numbers in fetal liver are presented in . At day 140 of gestation, hepatocyte number ($P<0.05$) and hepatocyte nuclear diameter ($P<0.01$) in the R1 group were significantly

or extremely significantly lower than those in the C group, while endothelial cell number was significantly higher ($P<0.05$), with no significant difference in Kupffer cell number between the two groups ($P>0.05$). The R2 group showed significantly higher endothelial cell number compared to the C group ($P<0.05$), with no significant differences in hepatocyte number, hepatocyte nuclear diameter, or Kupffer cell number ($P>0.05$).

2.3 Effects of IUGR during Late Pregnancy on ECM Content in Fetal Liver

The effects of IUGR during late pregnancy on fetal liver ECM are shown in . At day 140 of gestation, contents of collagen type I ($P<0.01$), collagen type III ($P<0.05$), collagen type IV ($P<0.01$), laminin ($P<0.01$), hyaluronic acid ($P<0.05$), and fibronectin ($P<0.01$) in the R1 group were significantly or extremely significantly lower than those in the C group. The R2 group exhibited significantly lower fibronectin content compared to the C group ($P<0.01$), with no significant differences in collagen type I, collagen type III, collagen type IV, laminin, or hyaluronic acid contents ($P>0.05$).

Discussion

Normal growth and development of an organism is manifested through normal cell proliferation, including increases in cell number and volume. As an important endocrine organ, the liver's basic structural unit is the hepatic lobule, which is primarily composed of hepatocytes accounting for 75% of the lobule volume. Other cells within the hepatic lobule are collectively referred to as sinusoidal cells, including endothelial cells, hepatic macrophages, large granular lymphocytes, and hepatic stellate cells. Cells are the fundamental units of organism and organ structure. Research has demonstrated that IUGR severely affects fetal body weight and liver weight, leading to significant reductions in cell size and number. Maternal nutritional restriction results in insufficient energy supply to the fetus, inevitably affecting fetal weight and liver growth, developmental regulation, and physiological function. Under the conditions of this experiment, severe restriction significantly limited fetal body and liver weight gain in both R1 and R2 groups, with impaired liver development consistent with previous reports. The impact of decreasing nutritional levels on fetal liver tissue cell proliferation and volume enlargement may be the primary cause of reduced fetal body and liver weight in IUGR fetuses. The significantly or extremely significantly lower hepatocyte number and nuclear diameter in the R1 group compared to the C group indicate that IUGR during late pregnancy reduces the proliferative capacity of fetal hepatocytes, obstructs cell volume enlargement, and alters fetal liver growth trajectories to varying degrees, which may be an important reason for decreased hepatocyte number and volume. Additionally, both R1 and R2 groups showed significantly higher endothelial cell numbers than the C group. Since hepatic metabolic function is primarily manifested through hepatocyte function, liver injury stimulates hepatocytes and Kupffer cells to

secrete endothelial cell-specific growth factors that promote hepatic sinusoidal endothelial cell proliferation. These findings indicate that increasing maternal restriction progressively affects fetal liver structure and function, consistent with previous research.

ECM serves not merely as a supporting tissue but also as a site for nutrient supply and immune response, adapting to external environments and maintaining internal homeostasis. ECM is primarily synthesized and secreted by hepatocytes and endothelial cells, comprising collagen proteins (types I, III, and IV), proteoglycans (hyaluronic acid), and glycoproteins (laminin and fibronectin). Collagen provides mechanical support for organisms, maintains organ and tissue integrity, and ensures normal functional performance. Type IV collagen and laminin jointly constitute the basement membrane, facilitating substance exchange between ECM and the external environment. Hyaluronic acid can prevent the production of certain enzymes in cells, reduce free radical formation, and protect cellular structures from free radical damage. Laminin plays a decisive role in embryonic development, and its reduction may underlie certain disease pathogeneses. ECM components are closely related to various cellular life activities, including cell growth and differentiation, and play crucial regulatory roles in cell proliferation and differentiation. Under the conditions of this experiment, the R1 group showed significantly or extremely significantly lower contents of collagen types I, III, and IV, laminin, hyaluronic acid, and fibronectin compared to the C group, while the R2 group exhibited significantly lower fibronectin content, indicating that nutritional restriction during late pregnancy reduces fetal hepatocyte number, leading to decreased ECM synthesis. Laminin plays an important role in embryonic development, maintaining cell and organ morphology and regulating cell proliferation and differentiation, and is primarily synthesized by epithelial and endothelial cells. IUGR-induced reduction in hepatic endothelial cell number may be an important factor affecting decreased laminin synthesis. Changes in ECM and its components may influence adipocyte volume, number, and morphology in animals, suggesting that fibronectin is an important early monitoring indicator for ECM synthesis under energy restriction. Liver injury reduces ECM secretion; on one hand, nutritional restriction decreases ECM synthesis capacity, while on the other hand, fetuses initiate hepatic fibrosis repair responses. When ECM degradation exceeds synthesis capacity, the balance is disrupted, potentially leading to further fibrosis. Hepatic parenchymal cell damage activates and proliferates hepatocytes through paracrine pathways, forming hepatic fibrosis. Hepatic fibrosis is closely related to ECM secretion; in this experiment, laminin content did not increase significantly in severe fibrosis tissues, possibly due to reduced repair capacity. The laminin results are consistent with previous research findings.

Conclusion

Nutritional restriction of Mongolia sheep during late pregnancy severely limits fetal liver growth and development. At a metabolizable energy level of 0.175

MJ/(kg BW · ·d), fetal liver ECM synthesis is severely impaired and hepatic fibrosis repair responses are triggered. At a metabolizable energy level of 0.330 MJ/(kg BW · ·d), only fetal liver fibronectin content is altered.

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Note: Figure translations are in progress. See original paper for figures.