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Effects of maternal undernutrition during late pregnancy on the development and function of ovine fetal liver

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ABSTRACT

This study investigated the effects of maternal undernutrition during late pregnancy on the development and function of ovine fetal liver. Eighteen ewes with singleton fetuses were allocated to three groups at d 90 of pregnancy: Restricted Group 1 (RG1, 0.175 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$), Restricted Group 2 (RG2, 0.33 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$) and a Control Group (CG, ad libitum, 0.67 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$). Fetuses were recovered at slaughter on d 140. Fetuses in the RG1 group exhibited decreased ($P < 0.05$) liver weight, total antioxidant capacity (T-AOC), superoxide dismutase activity (SOD), cholinesterase (CHE), total protein (TP), globulin (GLB), and alanine transaminase (ALT). In addition, intermediate changes were found in the RG2 fetuses, including decreased liver weight, T-AOC and CHE ($P < 0.05$). In contrast, increases in fetal hepatic collagen fibers and reticular fibers, glutathione peroxidase (GSH-Px), malondialdehyde (MDA), nitric oxide (NO), nitric oxide synthase (NOs), monoamine oxidase (MAO), albumin (ALB)/GLB, aspartate transaminase (AST), and AST/ALT were found in the RG1 fetuses ($P < 0.05$). The RG2 fetuses had increased fetal hepatic collagen fibers, NOs and MAO ($P < 0.05$) relative to the control fetuses. These results indicate that impaired fetal hepatic growth, fibrosis, antioxidant imbalance and dysfunction were associated with maternal undernutrition.

1. Introduction

Intrauterine growth restriction (IUGR), often resulting from maternal undernutrition during pregnancy (Robinson et al., 1999; McMillen et al., 2001), is a significant cause of fetal and neonatal mortality and morbidity (Bernstein et al., 2000; Wu et al., 2006). Considerable epidemiological data indicate that the growth of the heart (Han et al., 2004; Bubb et al., 2007), lungs (Lipsett et al., 2006), and pancreas (Dumortier et al., 2007) in IUGR fetuses are retarded, and these organs experience structural changes and impaired function that strongly predispose the animals to the development of cardiovascular disease (Barker, 1997), respiratory illness (Maritz et al., 2004), diabetes and insulin resistance (Vuguin et al., 2004; Green et al., 2010) in later life. As the largest of the body's organs with the greatest number of functions, the developing fetal liver is sensitive to damage from both internal and external sources, including teratogens, infection and nutritional deficiencies (Hyatt et al., 2008). The impaired growth of fetal livers caused by maternal undernutrition has been observed (Bauer et al., 1995; Osgerby et al., 2002; Hyatt et al., 2002; Gao et al., 2009), and altered gene expression of the hepatic PRL-GH-IGF axis has been detected in prenatal and postnatal

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animals (Bauer et al., 1995; Hyatt et al., 2002, 2004, 2007). Although these data indicate that exposure to an adverse nutritional environment in pregnancy has a long-term impact on fetal or neonatal liver growth, additional studies are needed to ascertain whether reduced liver mass and the expression of growth factors in IUGR fetal sheep affect liver function and, indeed, whether such responses contribute to the developmental programming of poor health in later life (Hyatt et al., 2008). Therefore, the objective of the present study was to test the hypothesis that maternal undernutrition during late pregnancy impairs hepatic growth and is associated with liver dysfunction in IUGR ovine fetuses.

2. Materials and methods

2.1. Animals and treatments

All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals in China (The State Science and Technology Commission of China, 1988). This study is a companion study, and the details of animals, experimental design and detailed procedures have been presented previously (Gao et al., 2013). Briefly, when the maternal nutrition density during late pregnancy is lower than the ensured "threshold" level (0.33 MJ ME kg $BW^{-0.75}$ d^{-1}), it may lead to Mongolian ovine fetal pathological changes and the ability of compensatory growth of postnatal offspring is suppressed or even lost (Gao, 2006; Qi, 2007; Gao et al., 2007, 2008, 2009). According to the previous results, three groups comprising eighteen Mongolian ewes carrying singletons were allocated at d 90 of pregnancy: a severely restricted group (Restricted Group 1: RG1, 0.175 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$), a restricted group at the "threshold" level (Restricted Group 2: RG2, 0.33 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$) and a Control Group (CG, ad libitum, 0.67 MJ ME kg $BW^{-0.75}$ d^{-1}, $n = 6$). Second or third parity ewes were mated at synchronized estrus and had similar live weights (mean 52.82 ± 2.67 kg) during the d 90 of pregnancy. Pregnancies were confirmed by ultrasound scanning at approximately d 50 of gestation (Medison-SA-600, Shanghai, China). All animals were housed in individual pens, and chopped hay (mainly *Leymus chinensis*) was supplied ad libitum until d 90 of gestation. Based on the fact that the fetus is considered to achieve 80% to 85% of its final birth weight during the last two months of gestation (Robinson et al., 1999; Symonds et al., 2001), maternal undernutrition was imposed from d 90 to d 140 of pregnancy. At the beginning of restriction, the ME and chemical composition in the hay were measured (Table 1), and then the daily intake of the hay offered in RG1 and RG2 was calculated by the ewe body weight, nutrition value of hay, and the designed energy plane in the restricted groups. Restricted ewes were fed at 08:30 and 16:00 h each day, and the amount of feed offered was constant throughout the restriction period (Table 2). The ewes in the Control Group were offered feed at 08:30, 11:00 and 16:00 h daily (the feed refusals were approximately 10% of the total amount offered). The animals had free access to water and mineral mixture blocks (containing per kilogram: Ca, 15 g; P, 11.5 g; Mg as $MgSO_4 \cdot H_2O$, 1 g; Fe as $FeSO_4 \cdot 7H_2O$, 500 mg; Cu as $CuSO_4 \cdot 5H_2O$, 250 mg; Zn as $ZnSO_4$, 175 mg; Mn as $MnSO_4$, 100 mg; Co as $CoCl_2 \cdot 6H_2O$, 20 mg; I as KI, 40 mg; Se as $Na_2SeO_3 \cdot 5H_2O$, 1.5 mg; Yuantongweiye Co., Ltd., Inner Mongolian, China). All feed refusals were collected daily before feeding at 08:30, weighed and sub-sampled for chemical analysis.

Table 1
Composition of grass hay and the leftover hay during restriction period.

	Grass hay	Leftover hay
ME (MJ/Kg)[a]	8.90	–
DM (%)	88.42	91.99
CP (%)	10.09	9.27
EE (%)	4.34	2.72
NDF (%)	71.98	71.19
ADF (%)	35.82	36.60
ASH (%)	4.67	4.39
Ca (%)	0.57	0.68
P (%)	0.09	0.08

[a] ME, metabolizable energy; DM, dry matter; CP, crude protein; NDF, neutraldetergent fiber; ADF, acid detergent fiber, EE = ether extract; Ca = calcium; P = phosphorus.

2.2. Slaughtering procedures

All fetuses were removed at d 140 of gestation. The umbilical cord blood was collected, and the fetal BW and liver weights were recorded. Some of the liver tissue was snap-frozen in liquid nitrogen and stored at −80 °C. Tissue samples (approximately 1.0 cm^3) were harvested from the large lobes of the liver. After rinsing with phosphate-buffered saline (PBS, pH 7.4), portions of the fetal liver were immediately placed in paraformaldehyde fixative solution (0.1 mol L^{-1} 7.4). After fixation for at least 2 days, the tissues were dehydrated and paraffin-embedded, sectioned at 4–6 μm and stained with commercial kits of reticulin stain (D032, NJJCBIO, Nanjing, China) and Masson stain (D026, NJJCBIO, Nanjing, China) for microscopic examination. Thereafter, five images were obtained from each section. Each specimen was viewed under a standard microscope, and the total number of collagen fibers and reticular fibers were counted in 10 random high power fields (HPF, magnification 100×) by three observers who were blinded to the study groups and results. Data were expressed as the number of collagen fibers per field and reticular fibers per field.

2.3. Liver chemical components analyses

Fetal liver moisture was determined by freeze-drying to a constant weight (Christ, Alpha 1-4 lsc, German), and sample components were analyzed for chemical fat, protein and ash. The crude protein content was determined using the Kjeldahl method, and the values were converted to protein using the factor 6.25. The chemical fat content was determined as the difference in DM before and after extraction using ether. The ash content of the fetal liver was the residue left after ashing at 550 °C in a muffle furnace.