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Effect of intrauterine growth restriction during late pregnancy on the growth performance, blood components, immunity and anti-oxidation capability of ovine fetus

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ABSTRACT

This study investigated the effect of intrauterine growth restriction on the growth performance, blood components, immunity and anti-oxidation capability of ovine fetus during late pregnancy. Six ewes out of 36 Mongolian ewes were slaughtered at d90 of pregnancy to serve as an initial comparison group. The remaining 29 animals were allocated to three different groups: Restricted Group1 (RG1, 0.18 MJ ME kg $w^{-0.75}$ d^{-1}, $n=12$), Restricted Group2 (RG2, 0.33 MJ ME kg $w^{-0.75}$ d^{-1}, $n=9$) and Control Group (CG, ad libitum, 0.67 MJ ME kg $w^{-0.75}$ d^{-1}, $n=8$). At 140 d of gestation, 6 fetuses in each groups were removed to determine the body weight, measurements and the umbilical cord blood was collected to analyze the blood components, $CD4^+$, $CD8^+$ T cells, total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and malondialdehyde (MDA). The results indicated the fetal weight ($P<0.01$), body length ($P<0.05$), thoracic girth ($P<0.05$), abdomen circumference ($P<0.05$), curved crown-rump length ($P<0.01$), red blood cell count ($P<0.01$), hemoglobin ($P<0.01$), packed cell volume ($P<0.01$), $CD4^+$ and $CD8^+$ T cells ($P<0.05$), T-AOC ($P<0.01$), SOD ($P<0.05$) were decreased and mean platelet volume ($P<0.05$), red cell distribution width% ($P<0.05$), GSH-Px ($P<0.05$) and MDA ($P<0.05$) were greatly enhanced in RG1 group. For RG2, a decrease of fetal weight ($P<0.01$) and T-AOC ($P<0.05$) was found in relation to CG group. With the decrease of the maternal dietary energy density, the fetal severe malnourished anemia, decreased immune capability and antioxidant imbalance appeared. These perturbations may have significant implications on postnatal growth and health, and will allow more effective predictions of fetal response to harsh conditions.

1. Introduction

Intrauterine growth restriction (IUGR) resulted from maternal undernutrition during pregnancy could lead to "programmed" alterations of physiological processes in the offspring (McMillen et al., 2001; Robinson et al., 1999), which would do harm to postnatal growth and health (Gluckman and Hanson, 2004; Wang et al., 2012; Warner and Ozanne, 2010) and induce an increased risk of perinatal morbidity and mortality (Sankaran and Kyle, 2009). A lot of researches indicated that these "programmed" perturbations might be the origins of a number of diseases in later life, including type 2 diabetes and insulin resistance (Green et al., 2010; Vuguin et al., 2004), high blood pressure (Gluckman and Hanson, 2004; McMillen et al., 2001), coronary artery disease (Barker, 1999) and respiratory illness

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(Maritz et al., 2004), and result in compromise of compensatory growth (Gao, 2006; Wyk, 2013). However, the further mechanisms, about the fetal origin pathological modification resulted from maternal undernutrition during late pregnancy, remain unclear. It is no doubt that the modification of fetal growth, development and health is the key factor for different growth trajectories in postnatal animal. Furthermore, as an important part of sustainable development in livestock production, the health of fetus is one of the most important productive processes, and decides the potential of production performance in postnatal animal. Therefore, the objective of this study is to investigate the effect of IUGR during late pregnancy on the ovine fetal growth performance, blood components, immunity and anti-oxidation capability.

2. Materials and methods

2.1. Animals and treatments

All experimental procedures were conducted in conformity with institutional guidelines for the care and use of laboratory animals in China (The State Science and Technology Commission of China, 1988). Thirty five Mongolian ewes in their second or third parity, which had similar live weight (mean 53 ± 3 kg) were mated at a synchronized estrus. Pregnancies were confirmed by ultrasound scanning at approximately 50 d of gestation (Medison-SA-600, Shanghai, PR China). Based on the fact that the fetus is considered to achieve 80–85% of its final birth weight during the last 2 months of pregnancy (Robinson et al., 1999; Symonds et al., 2001), maternal undernutrition was carried out from 90 d to 140 d of gestation. At 90 d of pregnancy, according to the slaughtering procedures outlined previously (Gao et al., 2008; 2009), 6 ewes were slaughtered to serve as an initial comparison group. The gravid uterine tissue fetuses were removed, followed by measuring of the fetal body weight and measurements. The fetal umbilical cord blood was collected in evacuated tubes containing lithium as anticoagulant for three purposes: the first sub-sample of 2 mL blood was used to analyze blood components; from the second sub-sample of blood, the lymphocytes were prepared to analyze the $CD4^+$, $CD8^+$ T cells, and the third sub-sample of 20 mL blood was centrifuged immediately at 3500*g* for 15 min and the plasma was stored at −70 °C for analyzing total antioxidant capacity (T-AOC), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and maleic dialdehyde (MDA). The remaining 29 animals were allocated to three different groups: Restricted Group1 (RG1, 0.18 MJ ME kg $w^{-0.75}$ d^{-1}, $n=12$), Restricted Group2 (RG2, 0.33 MJ ME kg $w^{-0.75}$ d^{-1}, $n=9$) and Control Group (CG, ad libitum, 0.67 MJ ME kg $w^{-0.75}$ d^{-1}, $n=8$). All animals were housed in individual pens and fed chopped natural hay (Table 1). Following 1 week acclimatization, the amount of feed offered was constant throughout the restricted period (Table 2). Restricted ewes were fed at 08:30 and 16:00 h each day. The ewes in 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 block. The ewes' body weights in each group were recorded at 90 d, 100 d, 120 d and 140 d of pregnancy. The feed refusals were collected daily and recorded before feeding at 08:30 and sub-sampled for chemical analysis. At 140 d of pregnancy, 6 fetuses in each groups were removed according to the method described above.

2.2. Blood components analyses

The hematological parameters such as white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red cell distribution width (RDW), platelet count (PC), mean platelet volume (MPV), platelet distribution width (PDW) and plateletcrit (PCT) were determined using an Automatic Blood Cell Analyzer (BC-2800, MindRay, ShenZhen, China).

2.3. MAb and flow-cytometric analyses

A 200 μl sample from 5 ml blood of each ovine fetus was taken and added to 2 ml erythrocytelysate; erythrocyte-depleted lymphocytes were suspended in phosphate-buffered saline (PBS, pH=7.4), then centrifuged at 1500*g* for 7 min at 4 °C. Lymphocytes (1×10^6) were prepared and washed twice with PBS. The lymphocytes were then stained with FITC-conjugated mouse anti-sheep $CD4^+$ mAb (MCA2213F, IgG2a), and PE-conjugated mouse anti-sheep $CD8^+$ mAb (MCA2216PE, IgG2a). All antibodies were purchased from AbD Serotec. FITC or PE-conjugated isotype-matched antibodies were purchased as controls (4ABIO, Beijing, China). Among them mouse IgG2a-FITC (FMCF002-100) and mouse IgG2a-PE (FMCP002-100) were included. Cells were incubated for 30 min at 4 °C. After washing, the cells were resuspended with staining buffer and analyzed on a Flow Cytometer (FacsCalibur, Becton Dickinson), and data were analyzed with Cell Quest software (Becton Dickinson).

2.4. Detection of T-AOC, GSH-Px, SOD and MDA concentrations in ovine fetus

The GSH-Px (A005), SOD (A001-1), and MDA (A003-1) were analyzed using commercial kits (NJJCBIO, Nanjing, China). They were determined using colorimetric methods with a spectrophotometer (WFJ 2100, UNIC instrument Co., Ltd., Shanghai) according to the procedures of Paglia and Valentine (1967), Panchenko et al. (1975), and Placer et al. (1966), correspondingly. The T-AOC (A015) was examined by commercial kits (NJJCBIO, Nanjing, China). The spectrometric method was applied to evaluate T-AOC. In the reaction mixture, ferricion was reduced by antioxidant reducing agents and blue complex Fe^{2+}-TPTZ (2,4,6-tri (2-pyridyl)-s-triazine) was produced. The absorbance was measured at 520 nm. One unit of T-AOC was defined as the amount that increased the absorbance by 0.01 at 37 °C. Data were expressed as unit per milliliter in serum.