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Metabolic profiles using ^{1}H-nuclear magnetic resonance spectroscopy in postpartum dairy cows with ovarian inactivity

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ABSTRACT

To understand the differences in metabolic changes between cows with ovarian inactivity and estrus cows, we selected cows at 60–90 days postpartum from an intensive dairy farm. According to clinical manifestations, B-ultrasound scan, rectal examination, 10 cows were assigned to the estrus group (A) and 10 to the ovarian inactivity group (B). All plasma samples were analyzed by ^{1}H-nuclear magnetic resonance spectroscopy to compare plasma metabolomic profiles between the groups. We used multivariate pattern recognition to screen for different metabolites in plasma of anestrus cows. Compared with normal estrous cows, there were abnormalities in 12 kinds of metabolites in postpartum cows with ovarian inactivity ($|r| > 0.602$), including an increase in acetic acid ($r = -0.817$), citric acid ($r = -0.767$), and tyrosine ($r = -0.714$), and a decrease in low-density lipoprotein ($r = 0.820$), very low–density lipoprotein ($r = 0.828$), lipids ($r = 0.769$), alanine ($r = 0.816$), pyruvate ($r = 0.721$), creatine ($r = 0.801$), choline ($r = 0.639$), phosphorylcholine ($r = 0.741$), and glycerophosphorylcholine ($r = 0.881$). These metabolites were closely related to abnormality of glucose, amino acid, lipoprotein and choline metabolism, which may disturb the normal estrus. The decrease in plasma creatine and the increase in tyrosine were new changes for ovarian inactivity of postpartum cows. The decrease in plasma creatine and choline and the increase in tyrosine and p-hydroxyphenylalanine in cows with ovarian inactivity provide new directions for research on the mechanism of ovarian inactivity in cows.

1. Introduction

As the number of large intensive dairy farms, and yield of dairy cows have increased, so too has the incidence of reproductive disorders in dairy cows [1,2]. In China, the morbidity from reproductive disorders is 20%–40%, with anestrus in 26.68%. Therefore, there is an urgent need to resolve the problem of anestrus in postpartum cows because of its influence on the reproductive performance of dairy cows.

Ovarian inactivity is a common type of anestrus in postpartum cows. This condition is characterized by growth of follicles only to the stage of follicular wave emergence, that is, up to ~8-mm diameter, after which, growth stops [3]. At 40 days postpartum, when uterus and ovary recover to normal physiology, there are no substantial changes in the follicular structures, accompanied by characteristic absence of a corpus luteum or cystic follicular structures [4].

Follicular growth needs various metabolites and environmental factors. This potential carryover effect of adverse conditions during oocyte growth and maturation on further fertility outcome was first proposed by Britt in 1992 [5]. Britt hypothesized that the developmental competence

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of the oocyte and the steroidogenic capacity of the follicle in high–yielding dairy cows are determined by their biochemical environment during a long period (up to 80 days) of follicular growth before ovulation [5]. Follicular growth is sensitive to metabolic signaling. Some research has indicated that changes in estradiol, GH, insulin-like growth factor-I, insulin, alanine, and glutamine can affect follicular growth and maturation [6]. Previous studies have not investigated the overall metabolic changes in healthy cows and cows with ovarian inactivity.

In recent years, as metabonomics technology has improved gradually, liquid chromatography tandem mass spectrometry, gas chromatography tandem mass spectrometry, and nuclear magnetic resonance (NMR) have been used to screen for diagnostic biomarkers of milk fever [7] and ketosis [8], and to compare the pathogenic mechanism of type I and II ketosis [9]. Postpartum ovarian inactivity in cows is affected by many factors, and the metabolic process is complicated and dynamic. Traditional 1-dimensional research cannot describe systematically and objectively the metabolic changes in cows with ovarian inactivity. Therefore, metabolomics ^{1}H-NMR technology was used to analyze the different metabolites in plasma of cows with ovarian inactivity and plasma of estrus cows in our study. We aimed to clarify the overall metabolic changes in cows with ovarian inactivity provide a theoretical basis for research into the mechanisms, prevention, and cure of ovarian inactivity.

2. Materials and methods

2.1. Animals

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. All experimental animals were treated according to the International Guiding Principles for Biomedical Research Involving Animals.

All cows were obtained from an intensive dairy farm in China. All diets were in accordance with the Chinese standards for cattle breeding. Cows were housed in intensive farming conditions with continuous access to fresh water and were milked 3 times daily. A total of 206 healthy Holstein cows with similar age and parity were selected as experimental animals. They were tracked to 60–90 days postpartum to observe the symptom of estrus and determine the follicle size by rectal examination and B ultrasound examination.

Normal estrus cows: spontaneous estrus at 60–90 days postpartum with obvious estrous signs. Rectal examination and B-ultrasound showed that there were no uterine and ovarian abnormalities and mature ovulatory follicles were present. The size of follicle was 15–20 mm by B-ultrasound.

Ovarian inactivity cows: a cow was considered to have ovarian inactivity when a follicular structure less than 8 mm was detected in 2 consecutive examinations, in the absence of a corpus luteum or cyst and no estrus signs were noted during the 7-day period between the examinations.

Ten estrus cows without other diseases were selected as the estrus group (A) and 10 cows with ovarian inactivity without other diseases were selected as the ovarian inactivity group (B).

2.2. Sample collection

Blood was taken in the 45–60 days of postpartum, when the estrus cows were in the stage of ovulation or will to be ovulation condition. At the same time, the blood samples of the cows which in the same postpartume days but had no follicle structure or the follicle size less than 8.5 mm were taken, if these cows have no follicle growth in the 60–90 days postpartum, the samples were as experiment group. If the cows estrus latter, the sample will be disgard. Samples of 10 estrus cows and 10 ovarian inactivity cows were taken finally.

Ten milliliters of blood was taken from the jugular vein of the 20 cows. The blood was placed in an anticoagulant tube with 3–5 drops of heparin sodium, and blood and anticoagulant were mixed evenly. Blood samples were centrifuged at 1500 $\times$ g for 5 minutes, the plasma were transferred to a 1.5-mL microcentrifuge tube, then the supernatant was centrifuged at 12,000 $\times$ g for 5 minutes, and 500-μL plasma was transferred to a 1.5-mL microcentrifuge tube, and stored at −80 °C.

2.3. Sample preparation

Before ^{1}H-NMR detection, all serum samples were thawed at room temperature. Aliquots of 200 μL were placed in 1.5-mL microcentrifuge tubes combined with 400-μL 99.8% D_2O buffer salt solution (pH 7.0) containing 0.2 mol/L Na_2HPO_4 and 0.2 mol/L NaH_2PO_4, 0.05% (w/v) sodium 3-(trimethylsilyl) propionate-2,2,3,3-d4. The mixture was centrifuged at 12,000 $\times$ g for 20 minutes at 4 °C, and 550-μL aliquots were taken into 5-mm NMR tubes (Wilmad) for detection.

2.4. ^{1}H-NMR spectroscopy

The NMR tubes that contained the 500-μL samples were placed under the signal acquisition probe of a 600-MHz digital NMR spectrometer (AVANCE III 500 MHz; Bruker, Switzerland). The NMR spectra were recorded using a Call–Purcell–Meiboom–Gill sequence, with 64 timed scans and a 10-ms total relaxation period (transverse and longitudinal relaxation) to inhibit the protein signal. The free induction decay signal was measured after transverse relaxation. All FID signals were multiplied by the exponential window function with a 1-Hz broadening factor before Fourier transformation to improve the signal-to-noise ratio, and then the ^{1}H NMR spectra were obtained.

2.5. Preprocessing of ^{1}H-NMR spectra

To obtain meaningful information from a mass of spectral data, dimensionality reduction ideology should be used to pretreat the data. The ^{1}H-NMR spectra were imported into Topspin version 3.0 software (Bruker, Ettlingen, Germany), and manually corrected, adjusting the baseline phase and phase position. The ^{1}H-NMR spectra were referenced to an internal α-glucose resonance at δ 5.23. The