Communication

iMedPub Journals
www.imedpub.com

Journal of Animal Research and Nutrition
ISSN 2572-5459

2021
Vol.6 No.5:94

DOI: 10.36648/2572-5459.6.5.94

Micrornas in the Serum of Pregnant and Non-Pregnant Cows

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Citation: Sowmya (2021) Micrornas in the Serum of Pregnant and Non-Pregnant Cows J Anim Res Nutr. Vol.6 No.5:94

Abstract

Circulating, non-coding RNAs, such as microRNAs (miRNAs) have been proposed to be powerful pathophysiological indicators of pregnancy in animals and humans. Since their discovery, it is known that miRNAs can take part in numerous biological processes, including cell proliferation and differentiation during early embryonic development and establishment of pregnancy. Our recent studies have indicated that maternal blood can carry miRNAs reported previously at the embryo–maternal interface in pigs. To expand the scope of our research, we tested the hypothesis that miRNAs previously identified in conceptuses, trophoblasts, endometrium and uterine lumen-derived extracellular vesicles (EVs) collected before Day 20 of pregnancy can show reproductive statusdependent profiles in the serum of cyclic and pregnant crossbred pigs. Customdesigned TaqMan arrays, multiplex real-time reverse transcription (RT)-PCR and real-time RT-PCR allowed us to identify.

Keywords: Plant clinics; Agricultural extension; Joint health services

Received: April 22, 2021, **Accepted:** April 28, 2021, **Published:** May 15, 2021

Introduction

Maternal recognition of pregnancy, embryo apposition, attachment and implantation followed by placentation are key events of early pregnancy leading to successful outcomes. Therefore, care and management of pigs in breeding herds at the early stages of pregnancy are essential. Unfortunately, pregnancy status in the pig can only be confirmed days after these key events are accomplished. It creates a unique niche to use circulating, non-coding RNAs as indicators of reproductive status, n large-scale random study (described below; Supplementary Figure S1), blood samples were collected at two other commercial breeding farms, recording differences in mean litter sizes (9.77 at farm A vs. 19.22 at farm B; January–June 2020), related to the known difference in prolificacy between Polish and Danbred [hybrid Landrace × Yorkshire] breeds. Blood (9 ml) was collected from pigs (n = 19; pure breeds of Polish Landrace, n = 3, Polish White Large, n = 5, both collected at farm A, and Danbred, n = 11, collected at farm B) on Day 15 after the secon AI. At Day 25 post-AI, ultrasound examination was performed to confirm pregnancy, which was further monitored until term to record litter size. Ultrasound examination revealed that 6 pigs were non-pregnant (Danbred, n = 5; Polish White Large, n = 1). Only 2 out of 19 pigs (Polish Landrace #70 and #197) were removed from the study due to hemolysis. Low RNA concentration (< 1.5 ng/µl) ob tained for two samples [1]..

AGO2 immunoprecipitates were separated with SDS-PAGE electrophoresis on gradient 4–15% gel (200 V, 40 min, 4 °C; Bio-Rad, Hercules, CA) and transferred onto a polyvinylidene fluoride membrane (1A, 25 V const., 30 min; Trans-Blot Turbo, Bio-Rad). After blocking antigens with 5% Blotting-Grade Blocker (Bio-Rad), blots were incubated overnight with polyclonal rabbit anti-AGO2 antibodies (1:500 dilution; Abcam, the same as for AGO2 immunoprecipitation) or with TrisBuffered Saline buffer, containing 0.1% Tween-20 for a negative control. Next, membranes were incubated with Immun-Star Goat Anti-Rabbit (GAR)-HRP Conjugate (1:20 000 dilution, Bio-Rad). Immune complexes were visualized using a Immuno-Star horseradish-peroxidase chemiluminescence kit (Bio-Rad). Imaging was performed using a VersaDoc MP 4000 and Quantity One 1-D version 4.6.9 software (Bio-Rad). To reduce viscosity and increase starting volume, 30 ml of 0.01 M PBS (137 mmol/l NaCl, 27 mmol/l KCl, 10 mmol/l Na2HPO4 and 2 mmol/l KH2PO4, pH 7.4) was added to 6 ml of each serum sample. Next, samples were stepwise centrifuged at 4 °C (2 000 ×g, 30 min; 12 000 ×g, 45 min) to remove any cell debris. The final supernatant was passed through 0.22 µm filters and ultracentrifuged at 110 000 ×g for 70 min at 4 °C in 10.4 ml polycarbonate bottles using an Optima L100 XP Ultracentrifuge (Beckman Coulter, Brea, CA; rotor 90Ti). Pellets from the same sample were pooled, resuspended in 0.01 M PBS (pH 7.4) and ultracentrifuged again at 110 000 ×g for 70 min at 4 °C [2].

The final EVs pellets were suspended in 100 µl of PBS and stored at − 80 °C for imaging EV samples were diluted 10 × with PBS, and 7 µl was loaded onto formvar-carbon-coated copper grids. Samples were stained with 1% uranyl acetate for 1–2 min and dried at room temperature. Images were obtained using a Tecnai 12 transmission electron microscopy (FEI), operating at an acceleration voltage of 100 kV, equipped with a CCD camera MegaView II [3].

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