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Short Communication

Impact of maternal physical activity during gestation on porcine fetal, neonatal, and adolescent ovarian development

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ABSTRACT

To determine how exercise from mid to late (days 40–104) gestation impacts offspring body, uterine and ovarian weight, and ovarian cell proliferation at three different developmental stages, Yorkshire gilts were either exercised by walking (EX) or not exercised (CON). In parity 1, ovaries and uteri were collected from the heaviest (H) and lightest (L) neonates and adolescent (6 mo) offspring. In parity 2, mothers were assigned the same treatment groups, and ovaries and uteri were collected from H and L fetuses on day 94 of gestation. Body weight was greater ($P < 0.02$) for H than L fetuses and neonates but not affected by EX treatment at any developmental stage. Ovarian weight in L but not H neonates was greater ($P < 0.02$) in EX than CON. Labeling index (LI; percentage of proliferating cells) was greater ($P < 0.01$) in cortex than medulla regions of fetal and neonatal ovaries. In fetal ovaries, EX enhanced LI ($P < 0.01$), and LI was greater ($P < 0.01$) in H compared with L offspring. In adolescent ovaries, LI was greatest ($P < 0.01$) in healthy antral and least in atretic antral follicles, and LI was greater ($P < 0.01$) in granulosa than theca cells of healthy antral follicles. Thus, exercise increased LI in fetal but not neonatal or adolescent ovaries. Although maternal exercise during gestation influences fetal and neonatal ovarian development, impacts on fertility remain unknown.

1. Introduction

Litter size in litter-bearing species has been demonstrated to have an effect on reproductive performance of the female. In sows, as size of the litter increases, blood flow to each fetus decreases [1]. This reduction in blood flow ultimately can cause detrimental effects on fetal development, thereby reducing reproductive efficiency and possibly fertility. However, exercise can enhance umbilical blood flow in pigs [2]. Furthermore, regular exercise may lead to enhanced umbilical blood flow at rest and potentially increase overall oxygen and nutrient delivery to developing fetuses [3]. Effects of maternal exercise on selected reproductive measurements have been investigated using several animal models including pigs, rats, and horses [4–9] and humans [3,10,11]. For pigs, it has been demonstrated that exercise did not have any effect on total number born but increased litter birth weight and greater survival to weaning age occurred [2,6,7]. Therefore, it seems that exercise did not have any detrimental effects on the reproductive performance of the sow and benefited the surviving piglets to their weaning age. However, differences in experimental conditions, such as duration and intensity of exercise and stage of pregnancy likely contributed to variable and sometimes conflicting results.

We hypothesized that an increase in blood flow to the fetus, caused by maternal exercise [2], would enhance ovarian and uterine development measured by organ weight and ovarian cell proliferation in offspring from three developmental stages. Therefore, the objective of this study was to determine how maternal exercise from mid to late gestation affects offspring body, ovarian and uterine weights, and cell proliferation in ovarian compartments at fetal, neonatal, and adolescent stages of development.

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2. Materials and methods

All animal procedures approved by the NDSU Institutional Animal Care and Use Committee (# A0927) were performed as previously described by Harris et al [2].

In experiment 1, Yorkshire gilts (parity 1) derived from the same sire and bred to a common boar (Hampshire × Duroc) were confirmed pregnant on day 28 of gestation. Individually penned (61 × 198 cm) gilts ($n = 4$ pairs) were assigned to treatment on day 36.1 ± 0.3 of gestation. Within a pair, one gilt was assigned to the control group (CON), which remained penned for the duration of the study, and the other gilt was exercised (EX) individually to avoid any social interactions. Exercise consisted of walking for 30 min three times per week from day 40 to 104 of gestation followed by a no-exercise period until farrowing. Within 15 h of the completion of farrowing, the ovaries and uteri were collected from the lightest (L; $n = 8$) and heaviest (H; $n = 8$) female piglets in each litter. Total uterine weight (without broad ligament) and ovarian weights were recorded for each piglet, and one ovary was fixed in Carnoy solution and one was fixed in formalin solution. The remaining piglets were allowed to grow until the age of 6 mo. One female from each litter was selected based on the adolescent status of her ovaries (lack of corpora lutea; $n = 8$) and her ovaries and uterus were removed, weighed, and fixed as described above.

Experiment 2 was initiated after 22 ± 3 d of lactation, where gilts ($n = 3$ in each CON and EX groups; parity 2) used in parity 1 were rebred to a common boar, placed on the same treatment as described in experiment 1 until day 94 of gestation. Then, sows were euthanized, and the H ($n = 6$) and L ($n = 6$) female fetuses were selected and their ovaries and uteri were removed, weighed, and fixed as described for experiment 1.

Formalin fixed ovaries were used for immunohistochemical procedures as described before [12]. To immunodetect Ki67 (a marker of proliferating cells) expression, ovarian tissue sections (one section taken from the middle of the ovary was evaluated for all stages of development; 4 μm thick) were treated as follows: (1) antigen retrieval in citrate buffer (10 mM; pH 6.0) in a microwave; (2) incubation with buffer containing normal horse serum (2%, vol/vol, ABC kit; Vector Laboratories, Burlingame, CA); (3) incubation with mouse monoclonal primary antibody (1:250; Vector Laboratories) against Ki67, for 1 h at 25°C; (4) incubation with a secondary anti-mouse antibody coupled to peroxidase (ImPress Kit; Vector Laboratories) for 1 h at 25°C; (5) incubation with Vector SG (Vector Laboratories) for 10 min; and (6) counterstaining with nuclear fast red to visualize the nuclei.

Image analysis was performed as described by Grazul-Bilska et al [12]. For each fetal ($n = 12$) and neonatal ($n = 16$) ovary, randomly chosen cortex (COR) and medulla (MED) regions (8–10 images per region) were generated using an Eclipse E600 Nikon microscope and digital camera (Nikon Instruments Inc, Melville, NY). For the adolescent ovaries ($n = 8$), images were taken from each of the 3 types of follicles, including secondary follicle (SF), healthy antral (HA), and atretic antral (AA; total 5–10 images/follicle/ovary/animal). For adolescent offspring born to CON and EX mothers, the number of SF, HA, and AA follicles analyzed was 19 and 21, 19 and 19, and 18 and 20, respectively. Follicles chosen for image analysis were selected based on the following criteria as described by Senger [13], SF containing the oocyte surrounded by two or more layers of granulosa cells. HA follicles containing a fluid-filled antrum housing the oocyte surrounded by many layers of granulosa and theca cells, and AA classified as an antral follicle with degenerated basement membrane, pycnotic nuclei, and detached granulosa cells. The images were then analyzed using the Image Pro-plus software (Media Cybernetics Inc, Silver Spring, MD) to determine labeling index (LI; the percentage of proliferating cells out of total cells per tissue area).

Results of measurements recorded for fetal, neonatal, and adolescent offspring were analyzed using the MIXED procedure (SAS Institute Inc, Cary, NC) with random effect being sow within treatment and fixed effects being treatment (CON vs EX) and weight (H vs L; fetal and neonate data). The model for the fetal and neonatal data included the effects of treatment, weight, and their interaction. Statistical analysis for LI of adolescent ovarian follicles (eg, granulosa and/or theca layer of SF, HA, AA) were analyzed using the general linear model procedures of SAS with fixed effects being treatment (CON vs EX). However, there were no treatment effects of the LI of adolescent ovarian follicles; therefore, the average was calculated for each follicle type. Means were separated using the LSMeans option of SAS and were considered significant when $P \leq 0.05$.

3. Results

Characteristics of the dam and litter have been previously published [2]. Data presented here are from offspring selected for determination of body, uterine, and ovarian weights, and LI.

At day 94 of gestation and birth, the H offspring were heavier ($P \leq 0.02$) than the L offspring (Table 1). Fetal and neonatal body weight (BW) was not affected by EX treatment. Because the H and L females from each litter were already selected at birth for neonatal analysis, adolescent females had similar BW regardless of maternal treatment.

Uterine weight at any developmental stage was not affected by offspring weight and/or maternal treatment. When expressed per BW unit, neonatal uterine weight was greater ($P < 0.01$) for L than H piglets (Table 1).

Ovarian weight (g or g/kg) was not influenced by EX treatment in fetal or adolescent offspring (Table 1). There was a treatment by weight interaction ($P < 0.02$; Fig. 1) where ovaries from the L neonates of CON dams were significantly lighter than ovaries from all other groups. When expressed per BW unit, there was a main effect of treatment ($P < 0.05$), with neonates from EX having heavier ovaries than CON sows (Table 1).

Fetal and neonatal ovaries contained primordial and primary follicles in COR and MED regions, and the adolescent ovaries contained primordial, primary, SF, HA, and AA follicles (Fig. 2). Expression of Ki67 was detected in COR and MED regions and in stromal cells and granulosa cells of primordial and primary follicles of fetal and neonatal ovaries and in stromal cells and primary, primordial, SF, HA, and AA follicles of adolescent ovaries (Fig. 2). In fetal ovaries, LI was greater ($P < 0.01$) in the COR (13.5 ± 0.9%) than MED