IDENTIFICATION OF BIOMARKERS FOR XENOESTROGENIC EXPOSURE USING A PREPUBERTAL PORCINE MODEL

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Background Information

- Zearalenone (ZEN) is a common mycotoxin detected in many cereal grains, especially corn, which are consumed by humans and domestic animals.
- ZEN is also a xenoestrogen which can interfere with normal endocrine function.
- Swine share many anatomical as well as physiological similarities with humans and other domestic animals.
- Porcine models are gaining in popularity for use in comparative biomedical research.
- The well-recognized susceptibility of prepubertal gilts to ZEN-induced hyperestrogenism and their physiological and anatomical similarities to juveniles of other species, underscore the suitability of the prepubertal porcine model for the identification and refinement of potential biomarkers for xenoestrogenic exposures.

Research Objectives

To enhance the utility of prepubertal porcine models by identifying additional novel biomarkers for xenoestrogenic exposures, in particular those ante- and postmortem biomarkers which are well-defined and measureable.

RESEARCH HYPOTHESIS

Morphometric biomarkers for exposure to xenoestrogens will be identified or refined in prepubertal gilts orally exposed to 0, 0.5, or 1 mg of zearalenone/kg of diet, for 21 days.

Specific Aims

Comparisons of the treatment group means for various measurements performed on stained sections of the uterus and ovaries, to determine relative sensitivities of these morphometric parameters as biomarkers for oral exposure of prepubertal gilts to low dietary concentrations of ZEN.

Acknowledgements

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Materials and Methods

- **Research Animals:** 18 prepubertal gilts were assigned to 3 treatment groups labeled A, B, or C. These gilts were orally exposed to either 0 (Group A), 0.5 (Group B), or 1 mg (Group C) of ZEN/kg of diet for 21 days.
- **Sample Collection:** Gilts were euthanized after 21 days of ZEN exposure and their reproductive tracts were removed and weighed, with sections of the vagina, uterine body, uterine horns, uterine tubes, and ovaries fixed in 10% neutral-buffered formalin and stained for histologic examination, using hematoxylin and eosin (H&E).
- **Histopathologic Evaluation:** Digital images of the urine body and ovaries were captured and selected parameters measured using SPOT (version 4.0.3) software. The width of the uterine submucosal and myometrial layers were measured, in micrometers, on several captured images (40X magnification) for each gilt (see Figure 1A). The number of primordial, primary, secondary, and tertiary follicles were counted in each of ten to twenty captured images (100X magnification) for each gilt (see Figures 1B and 1C). Given the lack of statistically significant differences between the mean percentages of primordial, primary, secondary and tertiary follicles in treatment groups A, B, and C, an ovarian scoring system was developed. A score of 1 was assigned to a gilt's ovaries which were devoid of tertiary follicles and any secondary follicles with an average diameter greater than 400 microns. A ovarian score of 2 was assigned to a gilt having at least one secondary follicle greater than 400 microns in diameter and/or a tertiary follicle with an average diameter measuring less than 2 mm (2,000 microns) on her ovaries. The ovarian score for a gilt was 3, if there was a single tertiary follicle exceeding 2 mm in diameter, and a gilt's ovarian score was 4, if there were multiple tertiary follicles with an average diameter exceeding 2 mm.
- **Uterine and Ovarian Caliper Measurements:** Calipers were used to measure the average diameter, in mm, of the uterine horns for each gilt and the average perimeter of the ovaries.
- **Statistical Analyses:** Utilizing SigmaPlot (version 13.0), the treatment group means for each of the measured uterine and/or ovarian parameters, as well as mean ovarian score for the gilts in a treatment group, were calculated and compared to one another using ANOVA and the Tukey Test for pairwise comparisons.

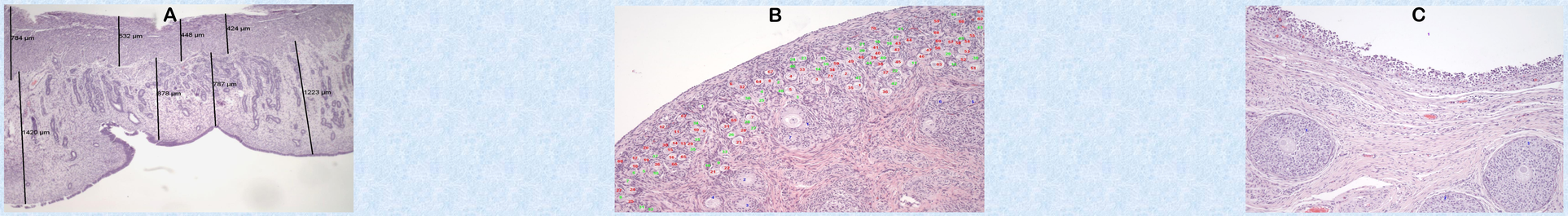

FIGURE 1: A section of uterine body (40X magnification), with measurements of the thickness of the submucosa and myometrium denoted is shown in A. Primordial, primary, secondary, and tertiary (antral follicles) are denoted by red, green, blue, and purple numbers, respectively, in B and C. Only a portion of a large tertiary follicle is shown in C.

Results

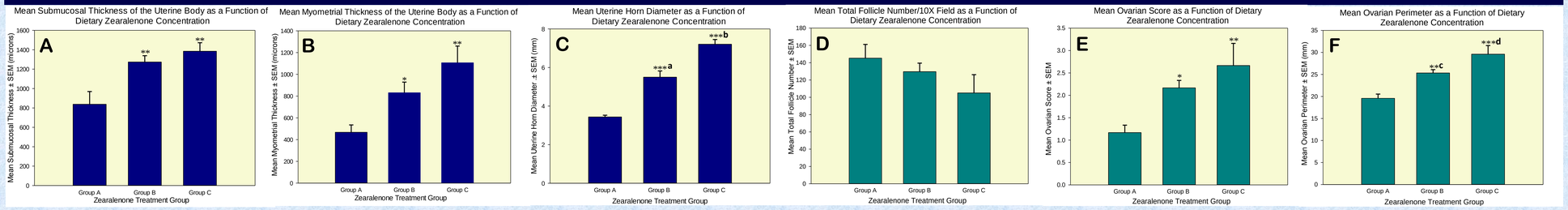

FIGURE 2: Uterine morphometric parameters are depicted in Graphs A, B, and C. Morphometric parameters pertaining to the ovaries are shown in Graphs D, E, and F. Asterisks are used to represents statistical differences between ZEN treatment groups B and/or C (0.5 and 1.0 mg ZEN/kg diet) and the negative controls (treatment group A). The greater the number of asterisks signifies, the greater the statistical significance (* = P-value >0.05 and < 0.10; ** = P-value >0.0001 and <0.05; and *** = P-value <0.0001. The lowercase letters, a and b for Graph C and c and d for Graph F, denote a statistical difference between treatment groups B and C (P-value <0.05 and P-value >0.05 and <0.10, respectively).

CONCLUSIONS

- **Morphometric parameters involving uterine horn and ovarian size increased with the level of dietary zearalenone exposure.**
- **Treatment group means for number of follicles and the relative proportions of different follicle types did not differ significantly from one another.**
- **An ovarian scoring system was useful in distinguishing between the treatment groups, with respect to differences in follicular development.**
- **Several of the selected morphometric parameters have potential for use in future experiments using the prepubertal porcine model as a comparative model for exposures of humans and/or other domestic animals to zearalenone and, potentially, other xenoestrogens.**