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Characterization of Mitochondrial Variation Between Oocytes Harvested from Lean and Obese Pigs

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The overarching goal of our work is to study the influence of mitochondrial-nuclear crosstalk on development of obesity and metabolic disease, and specifically, whether the mitochondrial genome plays a significant role in obese phenotypes and whether this role can be manipulated to control the development of metabolic disease. However, underlying this hypothesis is the assumption that there is indeed mitochondrial variation between lean and obese individuals and that resultant metabolic phenotype is not merely driven by environmental effects such as an unhealthy diet. My research question involved examining differences in mitochondria between oocytes collected from genetically lean and obese pigs to justify the pursuit of mitochondrial manipulation as a means to circumvent metabolic disease.

Experimental models were a Yorkshire pig (a lean, selectively bred, meat-breed pig) and a Mangalitsa pig (an obese, genetically unimproved, lard-type pig). Oocytes were used as the tissue of choice because current efforts in mitochondrial manipulation indicate intervention in the embryonic stage; additionally, paternal mitochondria are excluded from the fertilized embryo. Ovaries were collected from both animals at the time of commercial harvest. Oocytes were manually aspirated and placed in media. Cumulus-oocyte complexes (an oocyte and the surrounding cumulus cells that provide it with support and signaling) were morphologically graded to predict maturation and fertilization potential. Oocytes that have more than five complete layers of cumulus cells (grade A) are expected to be more successful at maturation and fertilization. Oocytes were then stained with brilliant cresyl blue (BCB) to predict general developmental competence. Oocytes that are BCB positive have higher developmental competence. MitoView™ Green, a fluorescent mitochondrial stain, was then added to visualize the mitochondria themselves. Images of each oocyte were captured and assessed using ImageJ®, an image-analyzing software. Finally, oocytes were denuded of cumulus cells and stained with Nile Red, a fluorescent stain that binds cellular lipids, to visualize lipid content of the cells. Images were again captured and analyzed.

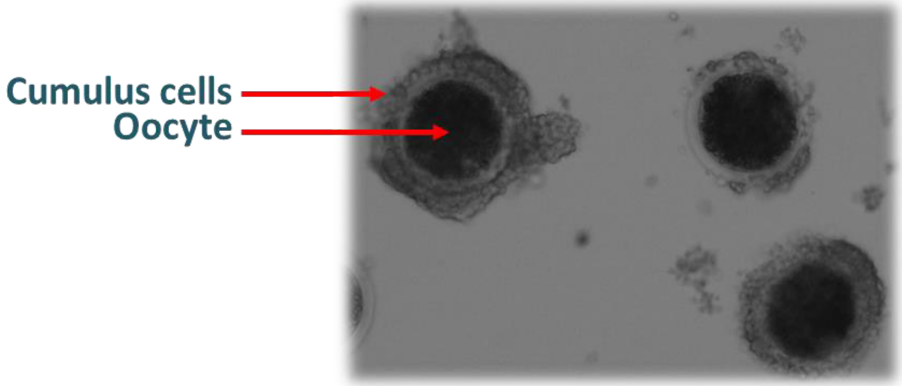
Oocyte grading showed that a larger percentage of Mangalitsa than Yorkshire oocytes were grade A, meaning that Mangalitsa oocytes had higher maturation and fertilization potential. BCB staining showed that a larger percentage of Mangalitsa than Yorkshire oocytes were BCB positive, suggesting that Mangalitsa oocytes were more developmentally competent. Mitochondrial staining showed that Yorkshire oocytes had stronger fluorescent signals (implying higher numbers of mitochondria) than Mangalitsa oocytes. Interestingly, BCB positive oocytes also had higher numbers of mitochondria than BCB negative oocytes regardless of breed, indicating that developmentally competent oocytes have more mitochondria, whether genetically lean or obese. Finally, Nile Red staining showed that Mangalitsa oocytes had higher overall lipid content as well as a higher ratio of polar to neutral lipids within individual oocytes. Neutral lipids are associated with fat storage within a cell, whereas polar lipids are associated with structural components.

Taken together, these data are consistent with the hypothesis that there is mitochondrial variation between lean and obese pig oocytes and that mitochondrial manipulation may indeed prove a worthwhile strategy to combat metabolic disease.

Statement of Research Advisor:

Maddy conducted an important pilot study to verify that differences in oocyte mitochondria exist between lean and obese pigs. Our overall aim is to examine the role of mitochondrial in obesity-induced metabolic disease. To accomplish this, we are pursuing a mitochondrial-nuclear DNA exchange strategy whereby mitochondria (and thus the inherent mitochondrial DNA) will be switched between embryos of lean and obese pigs. Maddy's results suggest that differences do exist between the mitochondria of Yorkshire (lean) and Mangalitsa (obese) pigs. Maddy's data are consistent with her hypothesis that disparities in mitochondria account in part for the divergent metabolic phenotypes exhibited across breeds. In doing so, Maddy has made an impressive and substantial contribution to the lab as Maddy's work provides critical support for further

pursuing mitochondrial nuclear exchange between Mangalitsa and Yorkshire pigs.
—*Terry Brandebourg, Animal Sciences*



Oocyte Grade	Layers of Cumulus Cells
Grade A	≥5 layers
Grade B	2-4 layers
Grade C	≤1 layer

***Figure 1**. Photomicrograph of cumulus-oocyte complexes (an oocyte and the surrounding cumulus cells that provide it with support and signaling) and table detailing grading criteria. Cumulus-oocyte complexes were morphologically graded to predict maturation and fertilization potential. Oocytes that have more than five complete layers of cumulus cells (grade A) are expected to be more successful at maturation and fertilization.*