Manipulation of Ovarian Function Significantly Influenced Glucose Metabolism in CBA/J Mice

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MANIPULATION OF OVARIAN FUNCTION SIGNIFICANTLY INFLUENCED GLUCOSE METABOLISM IN CBA/J MICE

by

Kyleigh Ann Tyler

Capstone submitted in partial fulfillment of the requirements for graduation with

UNIVERSITY HONORS

with a major in

Animal, Dairy, and Veterinary Science in the Department of Animal, Dairy and Veterinary Science

Approved:

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Logan, UT

Spring 2019
Abstract

Menopause is associated with a decline in overall health in women. One health aspect impacted is glucose metabolism. As women experience menopause, their metabolism declines dramatically. The current study addressed the influence of ovarian somatic cells on the improvement of metabolic health through transplantations of young, germ cell-depleted ovaries. The purpose of this study is to expand the understanding of female reproductive health on metabolism. Control mice were grouped by age and treatment mice were age-matched. Treatment mice were placed into one of three groups: 1) mice received germ cell-depleted ovaries, 2) mice received germ cell-containing ovaries, and 3) mice received ovarian somatic cells via injection directly to their original ovary. All mice were subject to a glucose tolerance test, during which a bolus of dextrose was administered, and blood glucose levels were collected and recorded. Mice were euthanized between 680 and 700 days. Metabolic results showed an improvement of glucose metabolism in both germ cell-depleted and germ cell-containing groups compared to controls. No significance difference was noted between the germ cell-containing and germ cell-depleted groups. Somatic cell injection groups also showed improved glucose metabolism compared to controls. This experiment has shown that post-reproductive health is positively influenced by reproductive status. Additionally, somatic cells play an important role in the restoration of health to post-reproductive mice.

Introduction

Menopause is defined as the period in women when menstrual cycles cease and is characterized by reproductive decline. Menopause is additionally associated with a sharp decline in many aspects of health. Before menopause, females hold a significant health advantage over their male counterparts; however, post-menopausal females experience an increased risk of disease compared to males of the same age. Menopause is associated with increased cardiovascular disease, increased risk of osteoarthritis, reduced muscular strength, and reduced cognitive ability.

Menopause is associated with a decreased metabolism and an increased risk for metabolic syndrome in a majority of women. Metabolic syndrome is characterized by increased weight gain and lipid deposition, insulin resistance, and low glucose metabolism. Existing treatments for menopause-related symptoms focus on hormone replacement therapy. Hormone replacement therapy, while effective at treating some symptoms of menopause, has not proven successful in the treatment of metabolic disease or in the treatment of other menopause-related health related changes (Lobo, 2008).

Ovarian failure is associated with the failure and depletion of ovarian germ cells. Ovarian somatic cells additionally play a central role in health. Previous studies showed that germ cell-depletion significantly improves skeletal muscle endurance (Habermehl and Mason, 2019). This experiment aims to evaluate the extent to which germ cells and somatic cells influence the metabolic decline associated with menopause. In current experiments, we chemically depleted ovarian germ cells in pre-pubertal ovaries and evaluated glucose metabolism in young and post-reproductive mice with and without ovarian germ cell-depletion. To further evaluate somatic cell influence on post-reproductive health, we conducted somatic cell injections into the ovary and evaluated glucose metabolism.
Materials and Methods

Female mice of the CBA/J strain were used in this experiment because they prematurely lose their ovarian follicles, leading them to become reproductively senescent at approximately 300 days of age (Thung et al., 1956; Jones and Krohn, 1961; Faddy et al., 1987). A reduction in the number of ovarian follicles in humans is associated with the onset of menopause, and thus was an ideal model in the study of age and menopause associate disease.

Female CBA/J mice aged 75 days, 200 days, 450 days, and 500 days were obtained from Jackson Laboratory (Bar Harbor, ME, USA). The mice were housed in individual, ventilated cages (Green Line IVC Sealsafe Plus, Tecniplast, West Chester, PA, USA). The laboratory environment included fresh, filtered air (15 changes/h), temperature (21 ± 2 °C, humidity of 50 ± 20%), a light-dark cycle (12:12h), and a specific, pathogen-free colony. Corn cob bedding (7097 Corncob, Harlan Teklad, Bartonville, IL, USA) was changed weekly. Cages also contained deionized water and added enrichments. Mice were supplied with a certified laboratory diet ad libitum (2018 Teclad Global 18% Protein Rodent Diet, Harlan Teklad, Bartonville, IL, USA). Mice were maintained in an American Association for Accreditation of Laboratory Animal Care (AALAC)-approved facility in accordance with the National Institutes of Health animal-use guidelines. Animal care and use protocols were developed under National Research Council guidelines found in the Guide for the Care and Use of Laboratory Animals. This project was approved by the Utah State University Institutional Animal and Care and Use Committee (IACUC-2277).

Anesthetics were used for both donor and recipient mice that underwent surgery. Analgesia was provided to recipient mice for 48 h post operation, or longer if deemed necessary. Donor mice were euthanized via cervical dislocation. A thoracotomy was performed immediately after cervical dislocation, followed by rapid exsanguination via cardiocentesis. Mice with acute weight loss were treated with moistened food and subcutaneous fluids. Mice with acute urine straining or rectal/vaginal prolapse were manually cleaned and treated with Desitin®. Mice were monitored at least twice a day and body weight was recorded monthly, more frequently if concerns arose. Moribund, aged mice that exerted overt clinical signs (catatonia) were euthanized. Criteria for euthanasia specific for aged mice were determined in coordination with the attending veterinarian and included, but were not limited to, mice found in poor condition with or without crusting around the perineum and diarrhea, urine staining, persistent vaginal prolapse, chronic vulva/rectal swelling, kyphosis, respiratory distress, anorexia, poor coat condition and lack of grooming, moribund mentation, hind-limb weakness/paresis, wounds not healing, limited mobility, neoplastic growth, and unusual weight loss (or gain). From peak weight to death, aged, female CBA/J mice have an average weight loss of approximately 12% per month (Mason et al., 2010). An increased rate of weight loss, but not total weight loss, was the most critical factor for determining a moribund state. Unexpected deaths were uncommon, but included neoplastic growths (most commonly mammary), decubitus ulcers (extremely old animals) and uncontrolled cataleptic seizures (normally 11-13 months of age). Mice that received germ cell-containing ovarian transplants lived on average 770 days. Mice that received germ cell-depleted ovarian transplants lived an average of 898 (Mason, et.al 2011). In this study,
control mice were euthanized at age 200, 300, 700, and 900 days. Treatment mice were euthanized at 700 days.

Experimental Design
400-day-old mice were randomly selected as control, germ cell-containing transplant recipients, and germ cell-depleted transplant recipients.

Controls
Control groups were created using the following criteria:
- 100-day-old mice that were reproductively cycling (n=19)
- 200-day-old mice that were both acyclic and cyclic during the study (n=10)
- 400-day-old mice that were acyclic (n=5)
- 600-day old mice that were acyclic and from a previous study (n=3)

Ovarian Transplant Groups
The ovarian transplant recipient groups were created using the following criteria:
- 400-day-old mice that were acyclic at the time of surgery and received 60-day-old ovaries from donor mice that were cycling (germ cell-containing) (n=9)
- 400-day-old mice that were acyclic at time of surgery and received 60-day-old germ cell-depleted (GD) ovaries from donor mice that were cycling (n=11)

Somatic Cell Injection Groups
Ovarian somatic cells were transplanted into the ovary using the following criteria:
- 400-day-old mice that were acyclic at time of surgery and received somatic cell injections to both individual ovaries. Somatic cells were obtained from 60-day-old mice that were cycling (n=5).
Figure 1. 28-day-old mice with young ovaries (YO) were treated with 4-vinylcyclohexene diepoxide (VCD) or Oil for 15 days. At 60 days of age, germ cell-depleted (GD) ovaries and germ cell-containing (GC) ovaries were removed and transplanted into 400-day mice. Unaltered ovaries from 28-day-old mice (YO) were digested and injected into the ovary and ovarian bursa of 60-day-old somatic cell injection recipient mice (SCI). Mice metabolism was evaluated via glucose tolerance test starting at 460 days. Mice were collected when treatment groups were 700±10 days.

Age at Manipulation

Normally, CBA/J female mice become sexually competent at 45-60 days of age. Manipulation was conducted at 28 days of age to avoid major up-regulation of the reproductive system at the onset of puberty and to eliminate influences the female gonad might have in addition to direct effects of gonadal hormones. Manipulation was conducted by injecting a placebo of sesame oil or treatment with 4-vinylcyclohexene diepoxide (VCD) to deplete germ cells from young ovaries (n=20). Germ cell-depleted donor mice received injections intraperitoneal of 160 mg/kg VCD once daily for 15 days. A previous 15-day VCD dosing protocol was created to stop reproductive cycling and deplete ovarian primordial and primary follicles (Mason, et al. 2009).
While rodents do not undergo menopause, they do have an estropause-like decrease in reproductive function. This decline in reproductive function begins at around 240 days of age and decreases gradually until reproductive incompetence at 330 days of age (Mason, Parkinson, Habermehl, 2018).

Surgical Procedures
Ovarian Transplants
Female mice of the CBA/J strain become reproductively active between 45-60 days old. At 60 days of age, cycling was confirmed in donor mice, and donor mice were anesthetized and had their ovaries removed. Cycles were confirmed using vaginal lavage. Donor mice were euthanized via cervical dislocation following removal of ovaries.

Recipient mice underwent ovarian transplant surgery and received donor ovaries as previously described (Mason, Cargill, Anderson, 2009). Briefly, mice were anesthetized, and a vertical incision was made in the lateral aspect of the abdomen. The ovarian fat pad was externalized, and a small opening was made in the ovarian bursa. The ovary was extracted and the donor ovary was placed within the empty ovarian bursa. The ovarian bursa was replaced within the body and the abdomen was sutured. The skin was stapled closed. This procedure was performed on both the left and right ovaries. All mice were allowed to recover for one month before metabolism was measured.

Injections
At 60 days of age, cycling was confirmed in donor mice, and donor mice were anesthetized and had their entire reproductive tract removed. Cycles were confirmed using vaginal lavage. Donor mice were euthanized via cervical dislocation following removal of ovaries.

Ovaries were isolated from the ovarian bursa from donor mice and placed in a glass convex dish. 50ul of digestion buffer (1% collagenase V, 100U DNase, 1% dispase with 290ul PBS+) was added, and ovaries were manually torn with 19 gauge needles for 5 minutes. Ovary solution was then placed into a 1.5mL Eppendorf tube. 100ul buffer was used to wash the glass dish and added to Eppendorf tube. Another 345ul digestion buffer was added to the ovary solution, total fluid within the tube at 500uL. Ovary solution was incubated at 37°C for 5 minutes with frequent pipetting, and 5 minutes with mild pipetting. 500uL of serum-containing media was added to the solution and allowed to sit for 5 minutes. The solution was spun down at 500g for 5 minutes. The supernatant was again removed, and pellet was resuspended with 1.0mL PBS-. The ovary solution was filtered through a 40um filter into a 50mL tube and spun down at 500g for 5 minutes. The supernatant was again removed, and remaining pellet was resuspended in 500uL of germ cell culture media containing a ZVF and AAV-GFP marker. Cells were plated in the first well of a four-well-plate and cultured overnight at 38°, 6% CO2. The next day, media was removed and deposited into the third well. The original well was rinsed twice with PBS/BSA, after which 100uL of 0.25% trypsin/EDTA was added and allowed to sit for up to 10 minutes. After cells have rounded, 400uL of germ cell media was added to the well and pipetted to a single cell suspension. 500uL was then added and suspension was incubated for 6 hours.
Pure somatic cells were created in the first well in this way and were used in somatic cell injections.

Female mice were injected with ovarian somatic cells as previously done. Briefly, surgical procedures to externalize the ovary were performed using the same operating procedure as ovarian transplants. Instead of removing the ovary, somatic cells were transported via a microinjection needle into the ovary and ovarian bursa. The ovary was then returned to the abdomen wall. This procedure was done for both ovaries. The abdomen was sutured closed and the skin was closed with skin staples. Mice were allowed to recover for one month before metabolism was evaluated.

**Exclusion Criteria**

Exclusion from analysis was based on gonadal input, defined as cyclic changes on vaginal cytology, presumably due to the cyclic influence of ovarian hormones. Absence of gonadal input was assumed to be indication of the lack of cyclic influence of ovarian hormones. Cycling was determined by vaginal lavage. Control mice that displayed cytological evidence of gonadal input 13 months of age or prior to surgery at 17 months of age were excluded from analysis. Ovarian transplant recipients that failed to display evidence of gonadal input postoperatively were also excluded from analysis.

**Metabolic Function-Glucose Test**

An intraperitoneal (IP) glucose tolerance test (GTT) was performed in mice that were feed-deprived for 4-5 hours previous. Fasting time was selected based on metabolic differences between mice and humans and due to aged female’s intolerance to overnight fasting (Ayala, et.al 2010). Blood glucose levels were measured using FreeStyle Freedom Lite Blood Glucose Monitoring System (Abbott Diabetes Care Inc. Almeda, CA, USA). Blood samples were obtained from a small nick at the tip of the tail, 2 h prior to testing and again immediately prior to glucose administration (t0), and at 15, 30, 60, 90, 120, and 180 minutes after injection. Glucose injection consisted of 20% D-glucose (2.8 g/kg lean body mass). Calibration of the FreeStyle Freedom Lite Blood Glucose Monitoring System was performed using control test solutions provided by the manufacturer.

Two drops of blood were collected, the first of which was discarded. The second drop was placed on the FreeStyle Freedom Lite test strip. Blood glucose reading was expressed as mg/dL. Glucose measurements at each point were then graphed and results expressed as Area Under the Curve (AUC).

**Statistical Analysis**

Statistical analysis was performed with GraphPad Prism 7.04 (GraphPad Software, Inc., La Jolla, CA, USA). D’Agostino-Person Omnibus test was performed to determine normality. Data were analyzed with a two-factor ANOVA and a Tukey-Kramer post-hoc test was used to determine differences between the groups. Student’s two-tailed t-test was performed on individual treatments assuming unequal distribution of variance. Test results were considered significant for P values P<0.05.
Results

Figure 2. Area Under the Curve (AUC) for young, old, age match, and very old control groups, and for germ cell containing (GC), germ cell-depleted (GD), and ovarian somatic cell injection (OSC) groups.

Figure 3. Blood glucose levels in mg/dL across time.
Blood glucose levels for each treatment and control mouse was mapped in mg/dL against time. Averages were also charted, as seen in Figure 3. At t=15 minutes, all mice groups experienced peak blood glucose levels. As seen in figure 3, Old Control mice on average peaked higher than other treatment and control groups after 15 minutes post-glucose injection. Additionally, the curve remains high as the glucose is metabolized, finally falling to similar levels to other groups after 90 minutes. The three other groups, Oil Control, Age Match Control, and GD Treatment groups follow similar curves. GD and GC both exhibited lower levels of blood glucose than the Age Match controls.

Young mice glucose metabolism exhibited a low area under the curve (AUC). As age of control mice increased, the area under the curve also increased. Very old mice (age 841 days) exhibited the highest area under the curve. Germ Cell-Containing (GC) Treatment group exhibited significantly lower AUC than the Age Match Control group (p < 0.012). Values were considered significant at p < 0.05. The AUC of the GD and GC was only slightly elevated over Young Control mice. GD mice, despite having an AUC below that of the Age Match controls, were not significantly different (p=0.3). There was no significant difference between the GD and GC treatment groups (p=0.06). While comparable levels, no significant conclusions can be drawn between these two groups.

Ovarian Somatic Cell Injection (OSC) Groups also exhibited lower blood glucose level peaks compared to Old Controls, Age Match Controls, and Young Controls. OSC did not have an AUC significantly lower than Age Match Controls (p=0.657). Additionally, OSC did not have a significant AUC compared to GD Treatment (p=0.343), nor did OSC have a significant AUC compared to GC (p=0.087).

Discussion

As females age, glucose intolerance increases. This intolerance can be characterized by a dramatic increase in blood glucose levels above fasting blood glucose levels after food consumption, as well as by an increased recovery time after food consumption for blood glucose levels to return to resting levels. A decrease in metabolic efficacy leads to other health problems, including metabolic syndrome and diabetes. These conditions can cause a decline in quality of life as well as overall lifespan. Loss of metabolic function has been previously associated with the loss of ovarian function in post-menopausal mice (Romero-Aleshire, M.J et. al, 2009).

Our control groups follow an expected trend following that of a metabolic curve in post-reproductive women. As the age of mice increased, so also did circulating blood glucose level and subsequent Area Under the Curve (AUC). Old mice exhibited a less efficient metabolic system, as can be seen both in Figure 2 and Figure 3. In both these Figures, the Area Under the Curve for old mice (age 841 days) is elevated beyond that of Age-Match Control (age 598 days) and Young Mice Control (age 168 days) groups. Elevated circulating blood glucose levels is a risk factor for disease and often leads to insulin resistance, leading to metabolic syndrome, and is seen in the old mice comparable to a naturally occurring menopause in women.

GD and GC groups both had reduced circulating blood glucose levels compared to their age match counterparts, suggesting that germ cells and somatic cells play a role in overall post-menopausal health. While conclusions are not statistically supported for GD vs. Age Match controls, the general trend may lead to an understanding about somatic cells in post-menopausal
health. The Somatic Cell Injection (OSC) group similarly had reduced levels. These results suggest there is a potential treatment option for menopause-related health decline directed at somatic cell therapy.

Currently, control of menopause-related symptoms has been addressed using Hormone Replacement Therapy (HRT). Few studies have been conducted on the effect of HRT on metabolism of post-menopausal women, but existing studies show mixed results and no proven treatment for metabolic syndrome or resistance (Lovre, 2017). HRT typically targets the hormone estrogen, which is decreased during menopause. Germ cells produce aromatase, which converts androgens to estrogens. Our research suggests that while germ cells may contribute to menopause hormone changes, these changes may not be exclusive in affecting overall metabolic health. Because both germ cell-depleted and somatic cell injection groups experienced improved metabolic health over old and age-match control groups, we propose that somatic cells also play a critical role in metabolic health. As a result, HRT may be less effective in correcting the decline in metabolic health due to the fact that somatic cells largely lack production of the hormones augmented by this therapy. We propose that ovarian somatic cell-based treatment may be more effective at treating metabolic decline than HRT. While this study lacked the direct evaluation of hormone, further studies will be conducted to test this idea.

Overall, young ovarian transplantation has a positive influence on glucose metabolism. GC groups confirm reduced blood glucose levels in post-reproductive mice. No statistical supported conclusions can be concluded for OSC and GC groups. In addition to this finding, somatic cell injections also had a positive influence on glucose metabolism. All treatment groups (OSC, GD, GC) showed reduced blood glucose levels.

Glucose metabolism is only one part of the complex metabolic system influenced by menopause. Continued studies in other aspects of metabolic health of post-reproductive mice have the potential to increase understanding of the roles somatic and germ cells. Future studies in this lab involve evaluating circulating triglyceride levels of GC, GD, and OSC mice to understand if germ cell-depletion influence triglyceride levels.

Conclusions

While women are living longer, the age of onset of menopause has stayed relatively constant. The relationship between somatic cells and germ cells in overall health is complex and still not well understood. Women are living longer with menopause and with the health decline associated with menopause. A health decline is associated with menopause that includes the decline in glucose metabolism. The decline in the efficacy of glucose metabolism may be improved with young ovaries that have been germ cell-depleted. Additionally, somatic cell injections proved to be successful in restoring glucose metabolism. This understanding has the potential to develop a somatic cell treatment for post-menopausal metabolism conditions. In developing existing understanding of somatic and germ cell communication, the hope is to develop a treatment to restore the health of post-menopausal women.
Acknowledgements

The author thanks Dr. Aaron Olsen, Mrs. Lisa Desoi, Kate Parkinson, Mckenna Walters for help with the mice. Additionally, the author thanks Utah State University, School of veterinary Medicine, Department of Animal, Dairy, and Veterinary Sciences, Utah State University Honors Program, and USUSA for funding and support. Finally, the author thanks Dr. Jeff Mason for assistance in project design,
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Author Biography

Kyleigh Tyler is a student in the Animal, Dairy, and Veterinary Science Department within the College of Agriculture and Applied Sciences at Utah State University, emphasizing in Bioveterinary Sciences. She has minors in chemistry, biology, and Leadership and Management. While at Utah State, she was involved as a University Community Engaged Scholar, College of Agriculture and Applied Science Student Council, and Undergraduate Research Fellows. Kyleigh has conducted and presented research throughout her time at Utah State. Her research has focused on health issues surrounding reproduction, and she plans to pursue this in her future educational endeavors. She will attend The Ohio State University School of Veterinary Medicine in Fall of 2019.
Reflective Writing

Kyleigh Tyler

My capstone project has significantly impacted and built upon my education at Utah State University. My path in research started my very first semester here, where I became involved with Dr. Benninghoff’s nutrition lab. After attending a guest lecture by Dr. Mason, I joined his lab which focuses on menopause, longevity, and reproduction. My capstone project combined these two experiences as I chose to focus on metabolic changes associated with somatic cells in ovaries. I was able to keep the contacts I had from Dr. Benninghoff’s nutrition lab as well as the knowledge I had learned in the development of my own project. My capstone project was effectively the perfect culmination of my education.

In addition to the knowledge I learned from participating in multiple labs, my research has helped me to apply the knowledge I’ve learned in my animal science classes. Because my capstone project has combined two major disciplines – nutrition and reproduction – these classes specifically have helped me understand the technical processes within my project. In addition, my endocrinology class was essential in developing a working knowledge of my project. There were many technical terms, processes, and procedures I had to learn. I was able to bring my unique experiences into the classroom. In understanding the details of these physiological processes, my classwork came to life and my knowledge was enriched.

There were some challenges that were presented in accomplishing my capstone. I finished experiments involving glucose early in this semester. Originally, I had planned to also analyze triglyceride deposits within our mouse serum and compare the two sets of data for one group of transfer mice. While the actual experiments were completed, analyzing data proved to be unrewarding. Despite my efforts and the efforts of those in my lab, our data was not showing
an expected trend. While the data was sound, the way we analyzed it was simply not working. Thus, my final paper is a reflection on glucose data from multiple groups of mice, which also showed an interesting trend. We have been working on analyzing this data in multiple ways, including pulling the opinions of different departments. We just recently, through collaboration and varying perspectives, discovered a flaw in within our Excel programing, and while I was not able to continue with my initial capstone plan, the research I’ve done can continue to grow. Being able to adapt was crucial to my project and was a valuable lesson learned in research. Often, research is unpredictable, and the ability to adapt is essential in a researcher. I learned how to overcome these challenges, which enriched my capstone experience.

The most rewarding part of my research was seeing the data give a staggering trend, a trend that has thus far been undocumented and has far reaching implications. Understanding the cells of the ovaries and how they influence health has the potential to help millions of people. To me, this is the most exciting thing about research, and something I’d like to dedicate my life to. I had no idea before entering college that such cutting-edge research was happening right here in Utah. My advice to future students is to be engaged in research that is happening, and don’t be afraid to spend time in different labs. My experience in two labs combined perfectly to lead to my capstone project, and both labs were conducting research I was interested in. Being able to both combine my passions and discover new passions was a highlight of my capstone project.

While Dr. Mason’s lab is under the Department of Animal, Dairy, and Veterinary Sciences, the students in our lab were diverse and from different backgrounds. Diversity within the lab helped me develop ideas and overcome challenges through collaboration. For example, two graduate students and a laboratory technician were available to teach me different procedures. Undergraduates students in the lab had majors in animal science, veterinary science,
and biology. Additionally, two vet students were periodically assigned to assist on projects. With so many different background readily available, multidisciplinary collaboration was easy and provided essential insight, especially in constructing my project. Collaboration was often facilitated during weekly lab meetings, where each of us brought current issues in our projects for discussion. Weekly meetings helped me to think critically and call on additional knowledge from all members of our labs.

One of the greatest experiences I had while conducting this research was the opportunity to share it with others within my community and beyond. This semester alone, I was able to present to State Representatives at the Utah State Capitol as part of Research on Capitol Hill, at the Western Regional Honors Conference held in Bozeman, Montana, and at the National Conference on Undergraduate Research held in Atlanta, Georgia. I was able to discuss my research with students from a variety of backgrounds, but often with students who shared a passion for human health. Research on Capitol Hill was a unique outlet for me to share my research with the community. I presented my research to representatives and to the general public of Utah. In this way, I had the opportunity to share my capstone with my immediate community.

Throughout my research experience, I have learned much about academia, careers in the field of veterinary medicine, and how to solve problems. While I knew coming into college that I wanted to become a veterinarian, I was unaware of the sheer number of roles a veterinarian could take. As I conducted research, specifically as I completed my own project, I was exposed to many of these roles. This realization of the far-reaching possibilities was perhaps the biggest benefit to my capstone project. As I attend veterinary school in the coming years, I plan to pursue a Master’s or PhD degree in addition to a DVM degree. Research has had such a
profound impact on my life. Creating my own project and engaging in research from start to finish has helped me decide to pursue a career in research as a research animal veterinarian. My capstone project was imperative in shaping this decision.
Germ Cell-Depleted Ovaries Improve Metabolic Health in Post-Reproductive Mice
Kyleigh Tyler
Utah State University

Menopause Definition
Period of time when menstrual cycles permanently cease due to the depletion of ovarian oocytes

Menopause Definition

Menopause

Pre-Menopause

Post-Menopause

Men

Women

Health Decline
Decline in Glucose Metabolism
Decline in Skeletal Muscle Mass
Decline in Cognitive Ability
Decline in Immunological Response
Decline in Longevity
Increased Muscular Tremors
Increased Lipid Dispositions and Weight Gain
Increased Osteoporosis

467 million post-menopausal women in the world
Ovarian Structure

- Germ Cells (Gocyte)
  Play a central role in reproduction
- Somatic Cells
  Supporting cells for the germ cells

Methods

- 4-vinylcyclohexene dioxide (VCD) injections once daily for 15 days prior to surgery
- 13-month-old, non-cycling females received 66-day-old ovaries
- 10 mice received GD ovaries; 9 received GC ovaries
- Mice received a bolus of intraperitoneal dextrose
- Blood glucose was evaluated at 0, 15, 30, 60, 90, and 120 minutes

Results

- Blood Glucose Levels vs. Time
- Glucose Metabolism
- AUC:

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Significance

- Somatic cell therapy to treat menopause symptoms
- Use of fibroblasts to adapt to humans
- Extend the lifespan and health of women

Acknowledgements

Mason Laboratory
- Dr. Jeff Mason
- Tracy Habermehl
- Kate C. Parkinson
- McKenna R. Walters
- Crystal Collier
- Anisa Samouri

USTAR/LARC
- Dr. Aaron Olsen
- Les DeBoo

Utah State University Honors Program