

8-2019

# Rescue and Reestablishment of Chicken Models for Spontaneously Occurring Hashimoto's Thyroiditis and Systemic Sclerosis/Scleroderma

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Rescue and Reestablishment of Chicken Models for Spontaneously Occurring Hashimoto's  
Thyroiditis and Systemic Sclerosis/Scleroderma

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Poultry Science

by

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University of Arkansas  
Bachelor of Science in Poultry Science, 2016

August 2019  
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This thesis is approved for recommendation to the Graduate Council.

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## **Abstract**

The loss of biodiversity is a topic gaining popularity both in the political and scientific forums. Nearly 30 years ago researchers and politicians congregated in Rio de Janeiro (1992) to attend the first Earth Summit. It was the first meeting of its kind discussing the tangible pressing consequences of biodiversity loss as well as the potential long term ramifications. Many of the countries represented at this summit implemented short and long term plans in order to accurately measure losses of biodiversity as well as establishing organizations to help diagnose and remedy the current problems at hand. These new organizations and researchers discovered that if biodiversity loss continued at the current rate both ecosystems and humans would suffer greatly.

It is known that planet earth contains billions of different life forms, a direct result of billions of years of evolution. Since the evolution of humans most, if not all, of these organisms have been able to co-exist harmoniously. It wasn't until recently that humans started to realize the detrimental effects of population growth and industrialization. In order to sustain all life forms changes must be made to salvage and sustain "at risk" populations included under the wide umbrella of biodiversity. Maintenance of biodiversity at all levels including agriculture and research populations will be an integral part of the solution. Utilizing and characterizing the genetic sequences that took billions of years to develop is of the utmost importance. The purpose of this study was to document the rescue and reestablishment of two avian biomedical research populations. These populations have undergone nearly 60 years of selective breeding and provide some of the top research models for studying their respective diseases.

## **Acknowledgements**

First and foremost I would like to thank my family and more specifically my parents, Tom and Ingrid Hiltz. Without your unconditional love and support I wouldn't be in the same universe as I am now. The spiritual, moral, and educational foundation you provided me has allowed me to lift myself up and pursue my true purpose. I would also like to thank my brother and sister for putting up with me throughout the years. Both of you have pushed me to be the best that I can be and I am forever grateful. Last but not least I want to say thank you to all of my grandparents. All of you have supported me every step of the way and made my collegiate aspirations a reality.

In January of 2016 I wasn't sure what direction my life was going. I want to thank Dr. Nick Anthony as he took a chance on me and offered me an assistantship that fall. I cannot express how grateful I am for everything that you have done for me. Thank you for allowing me to travel to Mozambique all these years, most professors wouldn't allow their students to do so. Thank you for your patience and unwavering support. Few students have mentors like yourself and I can honestly say you did nothing but promote and better the lives of your students. Professionally, personally, and spiritually speaking I don't know a better person in academia. Thank you.

A special thanks to Dr. Sara Orłowski. I can't say thank you enough for everything. It has been a pleasure working alongside you and I can't think of a better person to uphold Doc's program. Craig, it's been fun since we started in 2016 and I can't wait to see what happens these next few years. Thanks to Dave, Travis, Clay, Lucas, Lia, Will, Bradley, Chris, Matt, and everyone at the feed mill and farm. Without y'all none of this would be possible.

Thanks to Drs. G. Wick, R. Sgonc, H. Dietrich, Innsbruck, Austria and S. Kerje, Uppsala, Sweden for sending us fertile hatching eggs of both lines and their help with establishing the breeding populations at the University of Arkansas. Thank you to the Arkansas Biosciences Institute of Agriculture for the funding and support.

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## **INTRODUCTION**

Within the last 30 years substantial progress has been made in an effort to better understand how the loss of biodiversity affects both ecosystems and society (Cardinale et al., 2012). This was, in part, due to the first Earth Summit in Rio de Janeiro (1992) where scientists and politicians gathered to discuss the ramifications of biodiversity loss. As a result, a cascade of research initiatives formed and countries around the world started evaluating and collecting data on their respective environments. After years of collaborative research backing the newly reinforced theories surrounding the importance of biodiversity conservation, the scientific community embraced conservation efforts across the globe. The significance of the symbiotic relationships between biodiversity and ecosystem sustainability, along with human society are now being prioritized. A plethora of organizations and committees committed to the preservation, overall wellbeing of biodiversity, and the environment have emerged as a result of such events like the first Earth summit. These organizations include but are not limited to The Nature Conservancy, The Scientific Committee on the Problems of the Environment, The World Wildlife Fund, and the Intergovernmental Union for Conservation of Nature. Most recently in the news and at the top of many governments agendas are global conservation initiatives and the impacts of climate change on biodiversity and vice versa.

Biodiversity is often oversimplified by the majority of the public. Biodiversity by definition, is the collection of all plants, animals, fungi, and microorganisms inhabiting earth; as well as their genetic and phenotypic variation (Dirzo and Raven 2003). Biodiversity is often categorized into three levels; genetic, species, and ecosystem (Chen et al., 2004). Each of these three levels plays an integral part in the continuity and evolution of biodiversity across planet earth.

### **Systemic Consequences of Biodiversity loss**

Cardinale and colleagues (2012) demonstrated the potential impacts of biodiversity loss and the subsequent ramifications on humanity. Biodiversity is an extremely complex combination of abiotic and biotic components working together to create a functional ecosystem.

Cardinale and colleagues (2012) describe ecosystem services; “the suite of benefits that ecosystems provide to humanity.” This may be subdivided further into provisioning and regulating. “Provisioning services involve the production of renewable resources (for example, food, wood, fresh water). Regulating services are those that lessen environmental change (for example, climate regulation, pest/disease control)” (Cardinale et al., 2012). Often overlooked, these ecosystems provide tangible ecosystem services to humans. Cardinale and colleagues (2012) describes potential impacts of ecosystem services, “can a forest store more carbon if it has a greater variety of tree species? Can a stream clean up more pollution if it has a greater variety of microbial genotypes? Can natural enemies better control agricultural pests if they are composed of a variety of predators, parasites and pathogens?”

There are numerous studies showing the adverse effects of biodiversity loss pertaining to ecosystem structure and function. Most of these adverse effects fall into one of three categories; the influence of the physical formation of habitats, fluxes of elements in biogeochemical cycles, or the productivity of ecosystems (Cardinale et al., 2012). These categories often overlap in many ways due to the intricate and ecological relationships formed over billions of years of evolution.

The first category Cardinale and colleagues (2012) describes, involves the formation and influence certain organisms have on their respective ecosystem or environment. In the scientific community we call these organisms ecosystem engineers. The concept of ecosystem engineers is best explained by Coleman and Williams (2002) “Most engineering feats, according to the authors, separate along two lines: those produced by autogenic engineers whose morphological features alone precipitate local environmental change, and those produced by allogenic engineers, whose behaviors transform biotic or abiotic materials from one physical state to another.” A multitude of organisms have a distinct impact on the overall structure and functionality of any and every ecosystem in existence. A prime example of loss of diversity,

especially when considering human influence, is overfishing. Overfishing certain populations can have unforgivable consequences over time specific to their respective ecosystem.

Overfishing can lead to a trophic cascade, which is a direct interruption between predator-prey relationships within the food chain which can lead to a magnitude of indirect consequences for the whole ecosystem. Coleman and Williams (2002) also describe two top trophic level marine predators as perfect examples of ecosystem engineers. Both the tilefish and grouper are fish known to have an impact on both biotic and abiotic components of their respective environment, but due to overfishing in specific populations their respective ecosystems are at risk for trophic cascades. Coleman and Williams (2002) define the role and impact of these top level predators in their ecosystems, "As ecosystem engineers, their abiotic interactions leverage their ecological influence even further. This is particularly true if, by providing essential architectural structure in an otherwise less complex habitat, they support diverse communities of organisms. In addition, their burrowing behavior could potentially exert a major influence on sediment biogeochemistry and the breakdown and processing of deposited organic matter." Aforementioned was the idea of a trophic cascade resulting from the loss of biodiversity. This would be considered one of the three categories, more specifically the productivity of ecosystems. The overall productivity can be altered or affected in many ways but most simply the loss or drastic decrease of a keystone species such as the tile fish or grouper can lead to devastating changes in their respective ecosystem.

Another integral system of biodiversity affected by the loss of biodiversity is the distribution or fluxes of elements in biogeochemical processes. Biogeochemical cycles are essential to any lifeform on planet earth. It is how elements cycle through both biotic and abiotic pathways. Notable processes include the water, carbon, and nitrogen cycles. A countless amount of organisms play systemic roles in these respective cycles and greatly altering the number of these organisms could pose huge environmental consequences. Perhaps one of the more well known prokaryotes in ecology is Cyanobacterium. Cyanobacteria are the only known

photosynthetic prokaryote that have the ability to produce oxygen. These resilient bacteria are known to exist in almost every ecosystem around earth. These bacteria produce toxins which grow in eutrophic waters (Huisman et al., 2005). If these waters remain eutrophic and stagnant, opportunity arises for the bacteria to proliferate and bloom leading to the distribution of cyanotoxins. Current hypothesized climate change trends could have a direct effect on the proliferation of Cyanobacteria which in turn can not only lead to human health concerns but alter biodiversity in aquatic ecosystems. In addition to potential warming trends, “Human-induced environmental changes, most notably nutrient over-enrichment (particularly nitrogen and phosphorus) associated with urban, agricultural and industrial development, have led to accelerated rates of primary production, or eutrophication (Paerl and Huisman, 2009).” As a result cyanobacteria bloom and the toxins they produce are becoming a significant problem for human health and ecosystem biodiversity. Ingestion of many of the different cyanotoxins can lead to a multitude of health problems and in some cases death. In addition to human health concerns ecosystem biodiversity is also at risk. Frequent cyanobacteria blooms created by warming trends and eutrophic conditions amplify the already prevalent advantage of nutrient utilization given they bloom on the surfaces of water. Thus blocking light and nutrients to other phytoplankton as well as other aquatic species (Paerl and Huisman, 2009). As a result oxygen and carbon dioxide levels are altered as well as the aquatic ecosystems they reside in.

### **Agrobiodiversity**

Biodiversity is something often misunderstood and undervalued in the context of agriculture. Within the last 30 years technology has grown exponentially and we are now just understanding the genetic and phenotypic potential that lies within agriculture biodiversity. Although many organizations focus on wildlife and environmental biodiversity the importance of agriculture biodiversity cannot be stressed enough.

Current models predict that we will have to double the world food supply by 2050 through sustainable agriculture methods. The detrimental effects of climate change makes the

current challenge of sustainability ever more complex. The Food and Agriculture Organization of the United Nations (2019) also predicts that in developing countries where efficiency is needed the most, productivity will decrease between 20-40% due to the effects of climate change. Humanity will look to agriculture products and byproducts for medical, industrial, energy, and nutritional use (Scanes and Toukhsati, 2017). The biodiversity of agriculture has been influenced by natural ecosystems and vice versa ever since the inception of the agrarian revolution (Scanes and Toukhsati, 2017). Agriculture biodiversity also known as Agrodiversity, encompasses all animal and crop breeds and all of their wild relatives. It also includes the organisms associated with all other biological support systems including; microbes, pollinators, parasites, decomposers and pests (Jackson et al., 2005). Agrodiversity is positioned to be exploited through evolving molecular and quantitative techniques readily available to scientists. This presents an endless amount of creative solutions available to all aspects of human culture.

Agrodiversity is not only limited to food-based agriculture. Many plants and animals have significant roles in many different aspects of human culture (Scanes and Toukhsati 2017). Agrodiversity and the role it has played on human evolution and culture are immense to say the least. The transition from a hunter gather society to an agrarian has changed the course of human history. This involved some basic domestications of animals and plants. Most notably the domestication of cereals and other crops such as maize and potatoes (Scanes and Toukhsati, 2017). Agrodiversity is important for more than just food. In human culture animals have been a source of symbolism and often a source of religious significance (Scanes and Toukhsati, 2017). The use of many animals for fiber and animal skins have provided clothing and shelter for many of our ancestors as well as modern era fashion. Medical uses of domesticated animals are at an all-time high. Before any drug or medical treatment is used on humans, extensive research and testing must be performed on many different animal species. Animals also serve as biomedical models to help further our knowledge of certain medical conditions prevalent in humans.

The Food and Agriculture Organization of the United Nations (2019) estimates that approximately 7000 species of plants have been cultivated throughout the course of human history. This diversity is the result of direct and indirect selection pressures imposed by farmers or natural influences such as temperatures, soil conditions, pests, and diseases. Of these 7000 plant species, humans have utilized around 150 species for world commerce (Prescott-Allen and Prescott-Allen, 1990). According to the FAO only 30 crops/species provide 95% of human caloric requirements. Rice, wheat, corn, and potatoes alone, account for around 60% of global energy intake. As both human and natural selective pressures mound, it is of crucial importance to maintain high genetic diversity to ensure ample opportunities allowing for development of sustainable varieties of each respective species in respective environments. Global partnerships such as the Consultative Group on International Agricultural Research have created seed banks to preserve plant biodiversity around the globe.

Agrobiodiversity in domestic farm animals has followed many plant diversity trends. With commercial animal genetics companies hybridizing and selecting for specific efficiencies/traits, genetic diversity amongst popular farm animals has significantly decreased (Pinsenti et al., 1999). Scientists have warned commercial companies of such selection practices as certain genes/traits of commercially irrelevant low-production breeds are likely to contribute to future traits of interest (Notter 1999; Bruford et al., 2003; Toro et al., 2009). By allowing these low-production breeds to perish, the highly evolved specific traits such as disease resistance are also being lost. This is especially a problem in developing countries where conservationists are observing alarming rates of decreased biodiversity amongst the localized domestic animal breeds. Many of these breeds are being replaced by high production/efficient commercial breeds (Pinsenti et al., 1999).

In 2007, as part of a global collaborative effort, the FAO published an extensive report on the status and trends of animal genetic resources. Over 169 countries submitted reports to the FAO characterizing the current state of respective animal genetics and resources. The

report is titled “The state of the world’s animal genetic resources for food and agriculture.” Current livestock breed diversity is estimated around 7616 (FAO, 2007) and around 1491 of those breeds are classified as at risk. Of the 1491 breeds at risk 881 are mammalian species and 610 are avian species. Of the avian species at risk chickens make up the highest proportion at risk (30 percent). With regards to extinction, 643 mammalian breeds have been lost with cattle losing the most (203). Of the 11 avian species included in the report, 47 breeds have gone extinct with chickens losing 40 known breeds. The report excluded any commercially maintained breeds as well as breeds maintained by Universities.

### **Poultry Biodiversity**

The scientific community is now starting to appreciate the importance of poultry biodiversity/conservation given the demand for food and scientific research applications. Early geneticists such as William Bateson used poultry to demonstrate Gregor Mendel’s Laws of inheritance and epistasis (Fulton and Delany, 2003). Poultry as a nutritional source is highlighted due to the ever-growing human population and the demand for lean nutritious poultry products. “Poultry is kept in a wide range of agro-ecological zones and production systems, and under different economic regimes” (Hoffmann, 2005). Since the 1960s global per capita consumption of eggs has doubled, while poultry meat consumption has increased fivefold (FAO, 2019). Poultry meat leads global animal protein consumption making up 36 percent of the market. There are very few if any cultural restrictions on poultry products making it one of the few universal animal proteins. Given the projected population growth, poultry products will be an integral part of the solution to feeding the global population. As developing countries continue to grow and industrialize the demand for poultry will follow suit. Currently poultry is raised by 80 percent of households in developing countries (FAO, 2019).

Given the current and forecasted demand for poultry products there has been a growing concern given the decline of poultry biodiversity. Furthermore, the science and research application of many poultry breeds should not be overlooked. A considerable amount of the

current research lines are great models for many areas of interest in biology and the medical field. These genetic lines serve as important models for understanding immune system function as well other intricate biological processes, especially of human relevance (Pinsenti et al., 1999). Other breeds have been maintained to carry certain mutations affecting “specific developmental or metabolic processes providing a starting point for research exploring the tissue or cellular source of defects in embryonic development and metabolic function” (Pinsenti et al., 1999). Avian genetic lines also serve as valuable biomedical models for certain human congenital defects or disorders (Pinsenti et al., 1999).

According to the “The state of the world’s animal genetic resources for food and agriculture” report released by the FAO in 2007, there is about 2000 documented breeds of poultry in existence. Since 2000 there have been 47 known poultry breeds that have gone extinct, 40 of which were chicken. The report, however, does not include any populations maintained by commercial entities nor universities. A more detailed assessment focused on research lines in North America was compiled and released by Pinsenti and colleagues (1999) entitled the Avian Genetic Resources Task Force (AGRTF). This report was a status update as well as a proposal to create an Avian Genetic Resource center to maintain, develop, and distribute these valuable lines to any respective entity. The AGRTF reported the loss of over 238 research stocks between 1984 and 1998. Most of these stocks were unique research lines with no secondary populations and are considered lost. Years of collaborative research and rescue efforts gone. Of the total lines reported 40% of Canadian maintained lines were lost and over 60% of the U.S. lines were lost. Within the same assessment, the AGRTF reported 323 living stocks in the United States and 45 in Canada. Given this assessment is over 20 years old and subsequent budget/funding cuts, the assumption can be made that a notable amount of additional stocks have been lost.

It has been reported that more than 54% of said research populations in the United States are maintained at 6 locations (Siegel and Qureshi, 2006). This statistic illustrates the

ongoing global problem of consolidation and elimination of both Poultry Science departments and poultry research populations. Having such large proportions of these populations at few locations also poses other concerns. Disease challenges, natural disasters, and ongoing cost of maintenance pose huge threats the sustainability of these populations as a single event could have huge ramifications on a substantial proportion of the populations.

Poultry research populations have had contributions to our knowledge in three main areas; agriculture, basic life sciences (e.g. biology), and biomedical research. Poultry research stocks have been utilized as early as 1902 illustrating Mendelian inheritance and sex determination (Pinsenti et al., 1999). William Bateson, who characterized comb inheritance, was responsible for demonstrating that the same Mendelian laws that applied to plants also worked in animals (Delany, 1997). This perhaps was one of the pioneering experiments setting the stage for the use of poultry populations in scientific research applications.

Poultry research stocks have been maintained for many reasons over the past 100 years. Researchers have utilized avian species, specifically poultry, for a multitude of reasons. Poultry are a readily available animal well suited for a wide range of research applications. Many of the poultry species are prolific egg layers which provide researchers from different disciplines an opportunity to work/study with embryos and their development.

Besides some of the more technical advantages poultry provide, the most obvious reasoning for maintaining these research lines would be for agriculture purposes. Poultry products haven't always been a staple in America diets. In the early 1900s consumption of poultry was considered a luxury and it wasn't until late 1940s that the modern broiler industry started to take hold. In 1960, 28 pounds of chicken were consumed per capita annually. That number has more than tripled by 2018 with Americans consuming approximately 93.6 pounds per capita (National Chicken Council 2018). To keep up with current and future demand, poultry geneticists have innovated selective breeding techniques and schemes to design one of the most efficient farm animal commercially available. Commercial pedigree stocks have been

selected primarily for production related characteristics; (e.g. growth rate, feed conversion, egg production, skeletal structure, and disease resistance) (Pinsenti et al., 1999). Due to superior modern poultry breeding structures and techniques, poultry geneticists have influenced mammalian commercial farm animal genetics programs. Poultry stocks are also known to be used in pilot programs to test new breeding methods or ideas before implementing them in other species (Pinsenti et al., 1999). Part of this is due to the short generation intervals many species of poultry exhibit. Quail reach sexual maturity in 6-8 weeks of age with short egg incubation times. This allows for 4-5 generations in the matter of 12 months thus providing valuable insight into selective breeding ideas that would take years to divulge in other species.

### ***Poultry Research Populations***

In addition to agriculture advancements, many poultry research stocks have had meaningful contributions in the field of biology. Perhaps one of the most notable institutions housing many of these poultry research stocks is the University of California-Davis (UCD). Ursula Abbott and Hans Abplanalp were two of the most distinguished researchers responsible for developing over 50 different chicken research stocks. Most of these stocks were specially bred to be highly inbred or mutant research lines. The high number of available research stocks at the UCD location have offered researchers ample opportunity to study the chicken immune system, genetic sequence, and the effects of inbreeding on different reproductive traits (Pinsenti et al., 1999). Major studies on “the effects of the major histocompatibility complex haplotypes on disease resistance and the characterization of the physiological parameters of a chicken genetic immune-deficiency syndrome” have been due to the many different lines UCD has created and maintained (Pinsenti et al., 1999). In addition to the vast number of studies carried out on the chicken immune system, Abplanalp’s Red Jungle Fowl and White Leghorn inbred lines were used to create reference backcross populations and provide baseline DNA for the Chicken Genome Mapping Project (Pinsenti; Delany et al 1999). Abbott also has developed and maintained numerous lines exhibiting 14 distinct mutations with defined phenotypes expressing

abnormalities pertaining to craniofacial, limb development and the integument (Pinsenti et al., 1999). These research lines prove to be an invaluable resource to the scientific community as developmental biology continues to be one of the most interesting areas of science given modern molecular technology (Pinsenti et al., 1999). Researchers can explore embryonic development and stem cell functions with models like these. The UCD researchers also have developed many mutated lines that are used as biomedical models for studies pertaining to certain human genetic diseases such as scleroderma, scoliosis, and muscle dystrophy. (Pinsenti et al., 1999).

Other chicken populations known for meaningful contributions to the field of biology and more specifically cytogenetics were developed at Cornell University (Bloom and Bacon 1985) and in Australia (Thorne et al., 1987). These three lines are formally known as cytogenetic variants. These chicken populations exhibit chromosomal abnormalities such as aneuploidy, polyploidy, translocations, and large insertions or deletions (Pinsenti et al., 1999). These populations have served as suitable models to study genetic molecular processes including; meiosis, qualitative inheritance, genetic recombination, linkage patterns, transcription regulation, and gene dosage effects (Pinsenti et al., 1999). The Trisomic line led to the characterization of linkage and chromosomal locations of the Major Histocompatibility complex with the single nuclear organizer region (Bloom and Bacon 1985). Delany and colleagues (1995) describe the mPNU genetic line “The mPNU line segregates for a large deletion in the nucleolar organizer region of chromosome 16, giving it a reduced number of rRNA genes. The mPNU line was used to establish the developmental threshold (i.e., lethal limit) for rRNA gene copy number for the first time in a higher vertebrate (Delany et al., 1995).” The CSIRO Triploid line developed by Thorne has made many contributions to the areas of meiotic error and the characterization of sex determination, gonadal differentiation, sex reversal, and polyploidy effects on growth and development (LIN et al. 1986; Thorne et al. 1987, 1988, 1991, 1997; Solari et al. 1991; Thorne and Sheldon 1991). Research using these chicken based research models has led to a better

understanding of aneuploidy in humans, most notably; Down syndrome (trisomy 21), Klinefelter syndrome (XXY) and Turner syndrome (X<sub>0</sub>). The conservation of these cytogenetic lines has led to a better understanding of these molecular processes and will provide useful animal models for years to come.

Often noted as one of the most intriguing poultry research stocks, the parthenogenic turkey line developed by M.W. Olsen at the USDA research center in Beltsville, MD. This turkey population exhibited one of the most fascinating phenomenon in biology. Parthenogenesis in simple terms is the embryonic development of an unfertilized egg (Parker et al., 2014). Initially, Olsen discovered that approximately 14% of eggs laid by the Beltsville small white stock developed parthenogenetically (Parker et al., 2014). After intense genetic selection Olsen reported that 30 to 50% of unfertilized eggs started embryonic development. Hatch results of earlier Beltsville small whites were not reported, and it wasn't until 1975 that Olsen reported that approximately 1% of the unfertilized eggs laid by Beltsville small whites hatched. All the poult that did hatch were male and grew to be fully functioning toms. This population was the only known poultry stock selected for parthenogenesis until Parker and colleagues (2014) conducted studies using Chinese painted quail.

### ***Poultry Biomedical Research Populations***

As discussed, poultry populations have had substantial contributions in many disciplines of biology. Often overlooked are the contributions made from the biomedical poultry research stocks. Poultry and, more specifically, chickens have manifested very similar conditions of certain congenital diseases prevalent in humans. Typically these stocks are developed through inbreeding techniques as well as by utilizing mutant type birds from specific research populations. Many established biomedical poultry populations have been lost due to budget cuts and consolidation of respective poultry and animal science departments. One of the research stocks lost is a biomedical model for cleft palate, a congenital defect known to affect 1 in 940 newborns and is rated as the second most frequent human congenital birth defect (CDC, 2006).

Research stocks maintained by the University of Saskatchewan exhibiting neurological mutations such as congenital quiver, lethal tremor, sex-linked paroxysm, and pirouette, were all eliminated (Fulton and Delany 2003). Since the last Avian Genetic resources survey in 1999 there has been minimal status updates regarding biomedical research stock preservation. This has caused concern for researchers and conservationists alike as some of these valuable poultry research stocks are likely to be gone. Currently there are 3 research stocks that provide biomedical models for autoimmune diseases including vitiligo, scleroderma, and thyroiditis (Pinsenti et al., 1999). There also is a line of inbred leghorn chickens that model congenital adolescent scoliosis (Rucker et al., 1986).

### ***Chicken Scoliosis Research Model***

Lewis Taylor from the University of California- Berkley, was responsible for developing a line of leghorn chickens that mimic many of the characteristics of human adolescent scoliosis. Scoliosis is an idiopathic disorder affecting the spine of children and adolescents. It is the most common spinal disorder in children and adolescents prevalent in 0.47-5.2% of the population (Koneieczny et al., 2013). Grivas and colleagues describe scoliosis “scoliosis is characterized by a side-to-side curvature of the spine  $>10^\circ$ , usually combined with a rotation of the vertebrae and most often a reduced kyphosis in thoracic curves (Grivas et al., 2003).” Chickens selected from scoliosis line 263 were some of the first birds used in early studies. Incidence in this original population was around 50%, but after intensive selection incidence of scoliosis in sexually mature birds was prevalent at 90% (Rucker et al., 1986). Incidence of scoliosis was characterized as a spinal curve greater than  $10^\circ$ . Genetic inheritance and expression were initially attributed to three major autosomal recessive genes. The expression of adolescence scoliosis in line 263 is polygenic in nature. Higher incidence of severe scoliosis in males can be attributed to a sex influence on the scoliosis phenotype, rather than a sex linkage influence (Rucker et al., 1986). This animal model was considered superior to other respective models

due to its natural expression. Other models require environmentally inducing scoliosis via adding aminonitriles or depriving the animal of certain trace elements (Rucker et al., 1986).

Genetic expression of scoliosis in humans is still under review and is expected to be autosomal dominant, X linked, polygenetic, or multifactorial (Wise et al., 2008). Although scoliosis in chickens is expected to be autosomal recessive the homogametic sex influence relationship would translate to humans, as the incidence of scoliosis is higher in human females. Phenotypically and morphologically the chicken model has more similarities than differences in relationship to human manifestation of scoliosis. Growth plates for the vertebral bodies in both the human and chicken model lack growth abnormalities and appear uniform. Posterior elements in the chicken model are relatively uniform and resemble human scoliosis. In both species the development of scoliosis cannot be attributed to a primary defect in bone development. Anatomically speaking there are few differences between the model and human manifestation of adolescent scoliosis. Like scoliosis in human, the scoliosis in the chicken model seems to be influenced by environmental factors with regards to timing of expression. This avian biomedical model has provided insight into the pathogenesis and gene expression of scoliosis in humans, as well as the characterization of dietary effects on disease expression in the chicken model (Rucker et al., 1986).

Mutant chicken populations have been selected and developed into some of the most comprehensively studied autoimmune biomedical models available. Autoimmune diseases arise when abnormal immune responses target the organism's own healthy cells (US Dept of Health and Services). Autoimmune disorders are thought to affect at least 5-8% of the world's population, with many scientists thinking it could be much higher (NIH 2005).

### ***Chicken Vitiligo Research Model***

Vitiligo is an autoimmune dermatological disorder affecting around 1% of the world's population. Vitiligo is characterized by the autoimmune destruction of the pigment cells in the skin known as melanocytes. This results in depigmented patches of the skin and in some cases

systemic depigmentation covering the whole body (Spritz, 2006). The Smyth Line of chicken is known to be one of the best biomedical models available for autoimmune vitiligo (Erf, 2014). The Smyth line is a population of chickens originally developed by Dr. J. Robert Smyth Jr. at the University of Massachusetts, Amherst. Smyth also developed the Brown line and Light Brown Leghorn line, both of which serve as controls. In 1971 a non-pedigreed hatch yielded one amelanotic bird that ultimately led to the development of the vitiligo biomedical model known as the Smyth line. Incidence of amelanosis of the next few generations was observed to be around 2%. Selections were made focusing on pigmentation loss, severity, and reproduction viability, yielding a preliminary pedigreed population of amelanotic chickens. In subsequent years Smyth continued selecting both populations of Smyth and Brown lines based on MHC type. As a result, he developed sublines from the original populations. Similar MHC haplotypes were discovered in the Light Brown Leghorn breed as well, leading to the development of a control line in addition to sublines with definitive MHC haplotypes. Due to the closure of the poultry research facility at the University of Massachusetts and the retirement of Dr. Smyth many of these sublines were eliminated (Erf, 2014).

The development of the vitiligo and respective control lines offers diligent control and treatment options rarely seen in similar biomedical models. The Smyth line was selected to be vitiligo susceptible with most of the birds expressing vitiligo. The Brown line was developed as a parental control. It also is considered vitiligo-susceptible but has very low incidence. The Light Brown Leghorn line was used as the vitiligo resistant control pigmentation bird (Erf, 2014). All three lines are MHC-matched ( $B^{101/101}$ ), allowing for tissue exchange and adaptive cell transfer studies, to fully address the immune system's role in this autoimmune disease. A recent study conducted comparing genetic expression of these lines, indicates that there are only a few genes responsible for the phenotypic expression of vitiligo seen in the Smyth line (Sreekumar et al., 2001). Incidence of vitiligo occurs in 70-95% of the Arkansas Smyth line. This is characterized by post hatch destruction of melanocytes in feather and choroidal tissue. Onset of

vitiligo in chickens is somewhat like that of humans. In humans, destruction of melanocytes typically is seen during adolescence and early adulthood. In the Smyth line of chickens onset occurs around 6-16 weeks of age indicating a similar timeline for expression (Erf, 2014). Severity of amelanosis is more prevalent in chicken than humans. Incidence of other autoimmune disorders such as uveitis, hypothyroidism, and an integumental feather defect are high in the Smyth line. This phenomenon is not novel to the Smyth line as it is not uncommon to see other similar autoimmune disorders accompanying vitiligo in humans (Erf, 2014). The Smyth and respective control lines offer many distinct opportunities to study the immunological mechanisms and genetic interplay of vitiligo. It serves as one of the premier biomedical models offered in poultry and will continue to provide a relevant model for years to come.

### ***Chicken Scleroderma Research Model***

Scleroderma also known as systemic sclerosis, is an autoimmune disorder known to affect 3 in 100,000 people (Jameson 2018). Scleroderma is best summarized by Erf (2014), “Scleroderma is a complex autoimmune connective tissue disease characterized by pathological remodeling of connective tissues. Clinical and pathological features in humans include microvascular alterations; perivascular inflammatory infiltrates and alterations involving cytokines with either pro- or anti-fibrotic activity; aberrant activity of innate immunity as well as the T and B cell compartments; presence of multiple autoantibodies; and, ultimately, widespread tissue fibrosis of the skin and several internal organs.” Scleroderma is a complex disease with genetic factors playing a central role in addition to environmental influences affecting onset and severity. Very few animal models resemble their respective counterpart better than the UCD 200/206. It is considered the top animal model available for scleroderma as it manifests almost all clinical, histopathological, and serological conditions seen in human scleroderma (Erf 2014).

The first signs of dermal fibrotic disease were observed by Dr. Paul Bernier in 1942, at Oregon State University. Later in 1977, Gershwin and colleagues (1981) developed the UCD 200 line at the University of California, Davis. Shortly after the development of the UCD 200, the

UCD 206, which exhibits a homogenous B-15 MHC, was created to serve as a control line. Due to the popularity and success from serving as a valuable biomedical model, a UCD 200 colony was established at the Innsbruck Medical University's Experimental Animal facilities in 1988. Shortly thereafter a colony of UCD 206 was established at the same location in 1993.

Both the UCD 200 and 206 are similar in time course expression. Chicks from both lines appear relatively normal for 10-14 days after hatch. After 14 days approximately 90% of chicks will start to exhibit preliminary gross abnormalities. This includes severe swelling and erythema of the comb. As a result the combs become necrotic and are lost (Erf, 2014). This phenomenon is known as self-dubbing. By 21-28 days of age 20-40% of chicks develop dermal lesions in the dorsal neck region. Dermal lesions are described as swelling, induration, and loss of feathers. Erf (2014) describes histological observations "Histological examination has shown early skin inflammation that is later replaced by fibrosis of the dermis and subcutaneous fat and muscle". Internal organs are also affected as well as renal arterioles (Erf, 2014).

Incidence of scleroderma in the UCD 200 was higher in the homogametic sex similar to humans with scleroderma. Preliminary studies suggest that the genetic defect accountable for scleroderma in UCD 200 is autosomal, recessive and exhibits incomplete penetrance (Gershwin et al., 1981). Recent genetic analysis utilizing QTL mapping located loci suggesting a relationship to disease predisposition on chromosome 2, 12, and 14 (Ek et al., 2012). Orthologs in humans are also located on chromosome 2 and are responsible for similar immune functions and modulatory roles of scleroderma in humans. IGFBP3 found on chromosome 2, is considered the first genetic link between human scleroderma and scleroderma in chickens. SOCS1, located on the QTL region of chromosome 14, is another gene responsible for immunological function and is thought to be another useful genetic link between the two species (Ek et al., 2012).

The UCD 200 and 206 have proven to be one of, if not the best animal model available for studying Scleroderma. Scleroderma in the UCD 200/206 populations manifests itself

expressing very similar phenotypic and genotypic characteristics observed in humans. These populations will help to further our understanding of the intricate inter-relationship between genetic susceptibility, immune system defects and environmental factors that are responsible for the development and expression of this disease (Erf, 2014).

### ***Hashimoto Thyroiditis Chicken Research Model***

Hashimoto's thyroiditis is an autoimmune disease characterized by the autoimmune destruction of the thyroid gland. About 2% of the general population is affected, with incidence in women being much higher (10-20 times) than men (Erf, 2014). There are few animal models available for Hashimoto's thyroiditis, one if not the best animal model is the Obese Strain chicken. It must be noted that this line is an avian model for Hashimoto's thyroiditis rather than obesity. This animal model has been well documented and has made notable contributions to the general understanding of autoimmune organ specific diseases (Erf, 2014).

Development of Hashimoto's thyroiditis is multifactorial in nature requiring genetic susceptibility and environmental triggers to become clinical (Chisiakov, 2005). During the onset of Hashimoto's thyroiditis antigen presenting cells such as dendritic and macrophages infiltrate the thyroid. This is typically a result of an environmental trigger (dietary iodine, toxins, viral infection, pregnancy) leading to abnormal function of thyrocytes and the production of thyroid-specific proteins. These newly formed thyroid-specific proteins are utilized as self-antigenic peptides presenting themselves on the surface of antigen presenting cells. After processing, the antigen presenting cells and the applicable autoantigens move from the thyroid to secondary lymphoid organs. Interactions between antigen presenting cells, autoreactive T cells and B cells result in the production of thyroid specific autoantibodies and effector T cells. The newly recruited B lymphocytes, cytotoxic T cells and macrophages infiltrate and proliferate in the thyroid forming large areas of lymphoid tissue in the thyroid. Inflammatory cytokines are also produced during this stage. The newly formed autoreactive T cells, B cells and antibodies illicit a

massive cascade of thryocyte destruction via antibody-dependent, cytokine-mediated, and apoptotic processes of cytotoxicity leading to a hypothyroid state (Chisiakov, 2005).

The development of the Obese strain can be traced back to 1955 when R.K. Cole noticed three pullets in a flock of Cornell Strain birds expressing an abnormal phenotype. These birds were obese, small in body size and had long silky feathers (Van Tienhoven and Cole, 1962). Two of the three pullets survived and were used as pedigreed breeders to establish the Obese strain. Early breeding of the Obese strain yielded low incidence of the documented obese phenotype. In 1966 after exposing embryos to testosterone, it was determined that autoimmunity was involved and the described disease in chickens was very similar to Hashimoto's thyroiditis in humans (Cole, 1966; Cole et al., 1968). R. K. Cole continued to maintain and select the Obese strain for increased incidence of the hypothyroid phenotype until 1995. Cole was able to create a population expressing the obese phenotype in nearly all offspring by the early 1990's. In addition to the Cornell populations, in the early 1970's a flock of Obese strain birds was established at the University of Innsbruck, Austria. This new population was denoted as OS-INN. In 1987 researchers at Cornell sent dozens of hatching eggs to Innsbruck to establish closed-bred populations of the Cornell OS line (OS-C) and Cornell strain (CS-C). Cole maintained both Cornell strains until his retirement in 1995. Additional sublines based on MHC haplotypes were created in order to characterize MHC/immune system interaction. It is unclear when the OS and CS populations at Cornell were terminated but until 2015 Innsbruck was one of two institutions maintaining the Obese strain.

Erf (2014) summarizes the early stages of spontaneous autoimmune thyroiditis that appears at two weeks of age (post-natal). Histological examination of Obese strain thyroids reveals extensive infiltration by mononuclear cells and germinal center formation. At this time autoantibodies to thyroid antigens (Tg) can be detected in the bird's circulation. This infiltration of mononuclear and proliferation of germinal centers results in the autoimmune destruction of the thyroid by 4-8 weeks of age. Incidence of autoimmune thyroiditis was initially favored by the

female sex but after years of selection the parity between the sexes has disappeared. It has been well documented that Hashimoto's thyroiditis is polygenic and very complex encompassing a broad range of system interactions and environmental influences.

Due to the complex interactions and manifestation of spontaneous autoimmune thyroiditis, sublines based on MHC haplotype were developed in order to characterize disease etiology and pathogenesis. These sublines were homozygous for MHC haplotype B5, B13, and B15. Initially two of the three sublines were thought to have a prominent role in expression of disease severity. The sublines with B13 or B15 haplotypes expressed a more severe hypothyroid status versus the B5 haplotype. The respective sublines were continued and selected based on desired phenotype. After multiple generations exclusivity of severe disease expression was not limited to the B13 or B15 sublines as previously thought. After selections the B5 subline expressed similar disease severity as the B13 and B15 lines thus downplaying the influence of MHC haplotype pertaining to the development of spontaneous autoimmune thyroiditis.

It has been documented that at a minimum four to five genes are responsible for the manifestation of spontaneous autoimmune thyroiditis. These genes can be categorized as major or minor genes. The major genes responsible for predisposition and onset are described by Dietrich and colleagues (1999), "The major genes encode for two independent aberrations: a general dysregulation of the immune system that entails a nonspecific hyperreactivity against numerous exogenous and endogenous antigens and predisposes to autoimmunity, and a primary, recessively inherited target organ defect (Neu et al., 1985; Wick et al., 1985), i.e., an increased susceptibility of the OS thyroid to autoaggression, which explains the restriction of the autoimmune process to a single organ." Minor genes are thought to play a regulatory role of major gene expression and are notably important in animals lacking a complete set of the major genes (Dietrich et al., 1999). More specifically minor genes regulating gonadal steroids have a modulatory role in the pathogenesis of hypothyroidism in the OS line. Before the continuous and

meticulous selection for the hypothyroid phenotype, it was documented that females were much more likely to develop the disease. Exogenous exposure to testosterone in developing embryos confirmed the role of gonadal steroids in the development of hypothyroidism in the OS line as embryos injected with testosterone had a much lower incidence of hypothyroidism compared to the embryos not treated (Gause and Marsh, 1986; Faessler et al., 1988).

The development of the Obese strain chicken model has provided researchers ample opportunities to not only study hypothyroidism but autoimmune organ specific diseases. There are few models more complete and well documented than the Obese strain model developed by R.K. Cole. Disease etiology and pathogenesis has been extensively studied in part due to the development and maintenance of this biomedical research model. As current molecular and genomic technologies advance the conservation and utilization of models like the Obese strain will provide unique opportunities to study autoimmune diseases for years to come.

### **Methods of Conservation**

As outlined earlier, many of these valuable poultry breeds/stocks are being lost at an alarming rate and as a result researchers and conservationists are utilizing different methods to rescue and maintain these genetic stocks. Conservation methods and efforts to maintain poultry biodiversity can be categorized into two approaches: *in situ* and *ex situ* (Sawicka et al., 2011). The *in situ* method is simply characterized by maintaining live populations of birds. While the *ex situ* method is achieved through cryopreservation of genetic material and the successful regeneration of the preserved genetic material (Sawicka et al., 2011). Both methods are important and should be used in conjunction with each other to conserve and maintain poultry biodiversity.

Maintaining avian genetic resources via the *in situ* method has proved to be the most dependable method of conservation especially for inbred and long term selected stocks (Pinsenti et al., 1999). Maintaining live populations allows careful management and reproductive strategies ensuring the conservation of delicate research stocks known to have little success

through alternative cryopreservation strategies. Most researchers prefer this strategy as it ensures the conservation of many avian genetic resources and provides a readily available population for research application. This strategy has been used historically and is well documented but due to many recent budget cuts and lack of new funding many of these live populations have been lost or greatly reduced. As a result researchers are developing and utilizing *ex situ* strategies in an effort to conserve these stocks.

*Ex situ* strategies are a substantial part of the long term solution to conserve avian genetic resources. New advances in technology are providing insight into many of the cryopreservation techniques many researchers are currently using. Semen cryopreservation and preliminary primordial germ cell methods have been developed in an effort to conserve poultry resources. Of the two methods semen cryopreservation has been well documented and reviewed (Pinsenti et al., 1999). The idea of successfully cryopreserved semen would prove to be an invaluable method to conserve the male gamete. The relative amount of genetic material stored at a low cost would be ideal. Transportation and maintenance would be minimal and threats such as disease outbreaks and natural disasters are not of great concern (Pinsenti et al., 1999). However, the general success of semen cryopreservation is relatively poor and has many challenges to overcome before it becomes a sustainable conservation practice. Cryopreservation methods are known to damage or greatly reduce sperm function and fertilizing ability. Fulton (2006) has reported that fertility rates are often <30% and many times provide 0% fertility depending on the genetic line. It is also worth noting that these studies used pooled semen thus giving opportunity for an under/over representation of certain males to contribute to the successful fertilization events (Birkhead et al., 1999; Donaghue et al., 2003). Mutant and inbred stocks known already to have reproductive shortcomings would also face additional challenges when utilizing a technique such as semen cryopreservation. In addition to the physiological obstacles facing the preservation of the male gamete, this approach only provides 50% of the genetic contribution of the respective line. At this time there is no way to preserve

the female avian gamete. The female carries the W chromosome as well as the entire mitochondrial genome (Fulton, 2006). This poses a sustainable area of concern in trying to conserve avian genetic resources.

Cryopreservation of primordial germ cells (PGCs) is another method of *ex situ* conservation. PGCs are the precursors of germline cells responsible for the production of adult gametes (Yu et al., 2018). In this method of cryopreservation PGCs are isolated from blood sample obtained from developing embryos. After isolation the cells can be cryopreserved or directly injected into a host embryo to create a chimera. If utilized successfully the newly formed chimera will generate a small proportion of offspring containing germ cells/genetic material from the original embryo. This method is in its early stages of development and has the potential to play a substantial role in the conservation of many endangered or at risk avian resources.

## **Conclusion**

The conservation of biodiversity is an integral part for a solution to a sustainable future on earth. The loss of biodiversity even at the smallest level has everlasting detrimental effects on ecosystems and the services they provide. Implementing sustainable conservation programs is challenging but must be made in order to save many at risk species.

At this time to ensure the conservation of many at risk and vulnerable avian populations, researchers must utilize *in situ* conservation methods as much as possible. Maintaining live avian populations is expensive but until other *ex situ* methods become more reliable and readily available *in situ* methods are the only way to sustain many of these valuable populations.

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## **Chapter 1: Rescue and establishment of chicken models for spontaneously occurring Hashimoto's thyroiditis and systemic sclerosis/scleroderma**

### **ABSTRACT**

Chickens selected for spontaneous and predictable development of autoimmune disease have contributed to the understanding of complex, non-communicable diseases. Two such research lines include the Obese strain (OS) developed at Cornell University and the UCD-200 originating from the University of California Davis. The OS is valued for studying spontaneously occurring Hashimoto's thyroiditis. The UCD-200 chicken line is the only model for spontaneously occurring fibrotic disease (systemic sclerosis/scleroderma) with similar symptoms as those observed for humans. Following their establishment as biomedical research models at Medical Schools in Austria (Innsbruck) and Sweden (Uppsala), US maintenance of the OS and UCD-200 lines was discontinued.

In 2015, urgent requests were sent from both Innsbruck and Uppsala to adopt and rescue these valuable animal models at the University of Arkansas. A relocation plan was implemented which included importing pedigreed hatching eggs. The eleven chicks hatched from the UCD line originated from 4 unrelated sires and 4 unrelated dams. The OS line hatched 36 chicks from 10 unrelated sires and 11 unrelated dams. A minimum of 90 healthy viable birds per line were generated from these parents. Purity of the offspring was characterized by MHC-typing with OS being *B-13* and the UCD segregating for *B-2* and *B-15*. To examine whether the lines retained all of their unique autoimmune disease attributes, both lines were characterized with respect to disease incidence, time-course of expression, severity and immunopathology. The UCD line has good fitness and clearly shows the self-dubbing phenotype predominantly in males. Although the OS phenotype is clearly observed, the OS line is very difficult to manage and reproduce. Both the OS and UCD populations are considered reestablished viable breeding populations. Future breeding selections will combine phenotypic

and molecular assessment with careful mating structure to ensure sustainable breeding populations of both avian models.

## **Introduction**

Poultry populations have had substantial contributions in many disciplines of biology. Often overlooked are the contributions made from biomedical poultry research populations. Poultry and more specifically chickens have manifested very similar conditions of certain post-natal multifactorial diseases prevalent in humans. Two such research lines include the Obese Strain (OS) developed at Cornell University and the UCD-200 originating from the University of California Davis. The OS is valued for studying spontaneously occurring Hashimoto's thyroiditis. The UCD-200 chicken line is the only model for spontaneously occurring fibrotic disease (systemic sclerosis/scleroderma) with similar symptoms as those observed for humans. Typically biomedical research stocks are developed through inbreeding techniques as well as utilizing mutant typed birds from specific research populations.

The UCD-200 was developed in 1977 by M.E Gershwin at the University of California Davis. This is the only known animal model for spontaneous occurring systemic sclerosis/scleroderma. Gershwin maintained the population until 1988 and after receiving confirmation of successful establishments of UCD-200 populations at medical schools in Austria, discontinued the population. The Obese strain was developed by R.K. Cole at Cornell University in 1960. In the early 1970's Cole shipped dozens of hatching eggs to Austria (Innsbruck) allowing researchers to establish a new population of Obese strain birds designated as OS-INN. In 1977 Cole sent additional eggs to Innsbruck in order to establish closed-bred populations of the Cornell OS line (OS-C) and Cornell strain (CS-C). Cole maintained both Cornell Obese strain populations until his retirement in 1995. Researchers at the medical school in Austria (Innsbruck) also shipped hatching eggs to the medical school in Sweden (Uppsala), facilitating a new population of Obese Strain chickens. Documentation pertaining to the status of U.S. Obese strain populations isn't available but between Cole's retirement in 1995 and 2015

these populations were discontinued leaving the medical schools in Austria and Sweden as the only known locations housing these respective models.

In 2015, the medical schools housing these unique research lines couldn't continue to maintain either research population. Researchers from both institutions contacted the University of Arkansas in an effort to relocate and reestablish the populations. Shortly thereafter both medical schools sent dozens of pedigreed hatching eggs to Arkansas. The hatches yielded a base population of eleven UCD and thirty-six Obese (OS) chicks. The eleven chicks hatched from the UCD line originated from 4 unrelated sires and 4 unrelated dams. The OS line hatched 36 chicks from 10 unrelated sires and 11 unrelated dams. The following chapter describes the methods used to rescue and reestablish two avian biomedical models.

## **Materials and Methods**

### ***General Rearing***

Jamesway incubators were used to hatch all chicks in this study. Both lines were raised in 3.66m x 3.66m floor pens until sexual maturity. Chicks were started on 24 hours of light for the first 48 hours post hatch. After 48 hours chicks received 8 hours of light until production age at which lights were increased to 14-16 hours a day. Chick starter was provided ad libitum in feed pans and water was supplied via nipple drinker lines. At 4 weeks of age chicks were fed a grower diet ad libitum. House temperature was maintained at 31-34 C° for the first week. House temperatures were decreased by 2-4 C° each week until 21 C. After phenotypic selections at 10 weeks the OS line were fed a grower T4 diet. The OS line requires thyroxine (T4) supplementation (0.5 ppm) to simulate normal thyroid function and facilitate reproductive performance. At 18 weeks the UCD line were fed a layer feed ad libitum and the OS were fed a T4 layer diet ad libitum.

### ***Pedigree***

The pedigree hatch received in 2015 yielded a base population of eleven UCD and thirty-six obese chicks. The eleven chicks hatched from the UCD line originated from 4

unrelated sires and 4 unrelated dams. The OS line hatched 36 chicks from 10 unrelated sires and 11 unrelated dams. Mating structure for F1 was designed to minimize inbreeding utilizing unrelated sire and dam combinations. After completion of mating structure birds were placed into pedigree cages and artificial insemination was used to replicate each respective line. The UCD line shows good fitness and were replicated without any challenges. The OS line was difficult to manage and reproduce. Females from the OS line laid consistently with supplementation of T4 but males from the line had poor semen quality and quantity. Due to the poor reproductive performance of the males in the OS line, five small consecutive hatches were implemented in order to generate a viable F1 generation.

### ***F1 Generation***

Hatches in 2016 yielded 74 UCD chicks and 98 OS chicks. All birds were raised in 3.66m x 3.66m floor pens until sexual maturity. After sexual maturity birds were sorted into floor pens based on sex. The OS line was supplemented with Thyroxine (T4) at four weeks of age. Previous literature has noted that thyroxine supplementation is given after breeder selections are made (Dietrich et al 1999). Premature T4 supplementation can suppress the hypothyroid phenotype leading to an inaccurate assessment of potential breeder hypothyroid status. Breeders for both lines were selected based on maintaining maximum genetic variability in subsequent generations. A total of 18 sires and 36 dams from the UCD line were selected and placed into pedigreed cages. The OS breeders were represented by 24 sires and 48 dams.

### ***F2 Generation***

Initial hatches in 2017 yielded poor results for the OS line while the UCD line continued to display its strong reproductive attributes. A total of 5 different hatches were set for the OS line compared to a pair for the UCD line. Artificial insemination was used to replicate both lines. The first 3 OS hatches were pedigreed and yielded a total of 74 chicks from 13 different families. Due to the poor fertility and chick yields pooled semen was used for the remaining 2 OS hatches. The fourth OS hatch produced 104 chicks from 31 dams and the fifth hatch produced

157 chicks from 37 dams. Only 2 hatches were needed to replicate the UCD line. The first hatch yielded 78 chicks from 26 families and the second hatch produced 68 chicks from 21 families. Like previous generations the F2 generation was raised in 3.66m x 3.66m floor pens up until selections and pedigree placement. OS birds were not supplemented with T4 until phenotypic selections at 10 weeks. Initial OS selections were made at 5 and 10 weeks based on phenotype. Desired phenotype is described as obese, small in body size and long silky feathers (Van Tienhoven and Cole, 1962). In an effort to further characterize both lines, purity of the F2 generation was tested by MHC-typing blood samples. The OS line was typed for a *B*-13 MHC haplotype and the UCD line was typed for homo/heterozygosity *B*-2 and *B*-15. After MHC analysis of both lines final selections were made based on a second assessment of phenotype. After rigorous final selections new breeder populations were established. The new UCD breeder population was represented by 15 males and 12 females. The new OS breeders consisted of 35 males and 65 females. Similar to previous mating structures, sire and dam combinations were set up to preserve maximum genetic variability in upcoming generations.

### ***F3 Generation***

There was a total of 4 hatches in 2018. Hatches 1, 2, and 4 were pedigreed and were used for the selection of breeders. Hatch 3 was used for characterization and experimental purposes. Hatch results were as follows; Hatch 1 95 UCD (12 families), OS 94 (22 families), Hatch 2 84 UCD (11 families) 59 OS (18 families) Hatch 3 79 UCD (pooled semen) 28 OS (pooled semen) Hatch 4 65 UCD (11 families) 33 OS (17 families). Similar to previous generations birds were raised in 3.66m x 3.66m floor pens. At 5 weeks OS birds were sorted based on severity of phenotype. These data were also used later for selections. At sexual maturity (10 weeks) birds from both lines were separated based on sex. Shortly after the birds were separated by sex the OS line was sorted by the hypothyroid phenotype previously described. OS birds not exhibiting a hypothyroid phenotype were removed from the population (7-10%). After these initial selections MHC typing was completed to confirm purity and evaluate

a potential relationship between disease severity and MHC haplotype. After analysis of MHC type and phenotype, final selections for breeders were made. The new breeders from generation F3 were as follows UCD 48 females, 43 males; OS 58 females, 60 males.

In an effort to further characterize each line body weights were taken weekly. In addition to body weights the skin from the dorsal region of the neck was measured using a digital caliper. In UCD birds exhibiting normal/healthy dorsal neck regions measurements ranged from 0.2-1.9 mm depending on age of the bird. Recorded measurements over 1.9 mm indicated some degree of lesion in the dorsal neck region. Combs of the UCD line were scored for necrosis/“self-dubbing phenotype” on a 0-2 scale. A score of 0 indicated a normal healthy comb, 1 indicated a partial necrosis, and a score of 2 indicated majority/full necrosis of the comb. As discussed MHC typing was completed on both lines to evaluate a potential relationship between MHC type and severity of phenotype (figure 6 &7).

## **Results and Discussion**

The most recent hatch results indicate a successful rescue and establishment of both chicken biomedical research models. Since the initial base population hatch in 2015 both lines have been carefully bred and selected to maximize genetic variability and manifest their respective clinical symptoms often observed in humans. Additional data was collected in 2018 further characterizing both populations. A timeline of each population’s progress can be observed in Figure 1.

### ***MHC Haplotype Characterization***

MHC haplotyping was completed in an effort to confirm purity of each lines offspring as well as to further characterize MHC type and severity of each respective phenotype. Blood samples were collected from each bird and were tested for desired MHC haplotype. The OS line was typed for a *B*-13 MHC haplotype and the UCD line was typed for homo/heterozygosity *B*-2 and *B*-15. This MHC characterization will facilitate new subline populations of the UCD 200 chicken model. The new sublines will have different MHC haplotypes including *B*-2/*B*-2, *B*-

2/B15, and B-15/B-15. This will help to further our understanding of the role of certain MHC haplotypes have on disease expression. Results of MHC halpotyping can be observed in Table 1.

### ***Body Weights***

As part of the characterization body weights were taken for both lines. Average weights for each respective week were calculated and plotted (Figures 2,3). The UCD line was used as a body weight control due to its normal thyroid status. For the first 8 weeks of age the OS average body weights are higher than the UCD weights. From 9 weeks of age and onwards the UCD line exhibits a higher average body weight when compared to the OS. This is most likely attributed to the hypothyroid phenotype of the OS line. Typically around 6-10 weeks the complete hypothyroid phenotype will manifest itself and ultimately slow overall growth and development of the OS line until birds receive proper thyroxine supplementation (Dietrich et al., 1999).

### ***Dorsal Neck Lesions***

Skin measurements taken in the dorsal region of the neck provide a baseline for dermal lesions often expressed in UCD chickens. These lesions are described as swelling, induration, and loss of feathers. In UCD birds exhibiting normal/healthy skin in the dorsal neck region, measurements ranged from 0.2-1.9 mm depending on age of the bird. Recorded measurements over 1.9mm indicated some degree of lesion in the dorsal neck region. Highest measurements taken were 20 mm in width but as the lesions healed width of lesions regressed to a normal neck measurement close to 1.0 mm. Examples of dermal lesions in the dorsal region of the neck can be observed in birds 2-4 weeks of age (Figure 4). 23 of the 272 UCD scored expressed dorsal neck lesions. Figure 5 shows distribution of dorsal neck measurements. Table 2 shows MHC type association of dorsal neck lesions in birds where these lesions were observed.

### **Comb Necrosis Scores**

Combs of the UCD line were scored for necrosis/“self-dubbing phenotype” on a 0-2 scale. A score of 0 indicated a normal healthy comb, 1 indicated a partial necrosis, and a score of 2 indicated majority/full necrosis of the comb. Examples of each score can be observed in Table 3. Figure 5 shows distribution of comb scores and respective MHC type. 48/272 lacked any comb necrosis, 141/272 scored a 1 indicating partial necrosis of the comb, and 83/272 scored 2 indicating a majority/complete necrosis (loss) of the comb.

### **Conclusion**

Chicken models selected for spontaneously occurring Hashimoto’s thyroiditis and systemic sclerosis/scleroderma have had significant contributions to the understanding of complex, non-communicable diseases, and autoimmune organ specific diseases. Contributions from both lines have been well documented since their establishments in 1960 (OS) and 1977 (UCD). As new technology is developed such research lines will serve as premier biomedical models for their respective diseases. Elimination of valuable research lines serves a disservice to science and humans alike. Two such research line are now considered viable established populations and are available to other entities for further research.

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Appendix

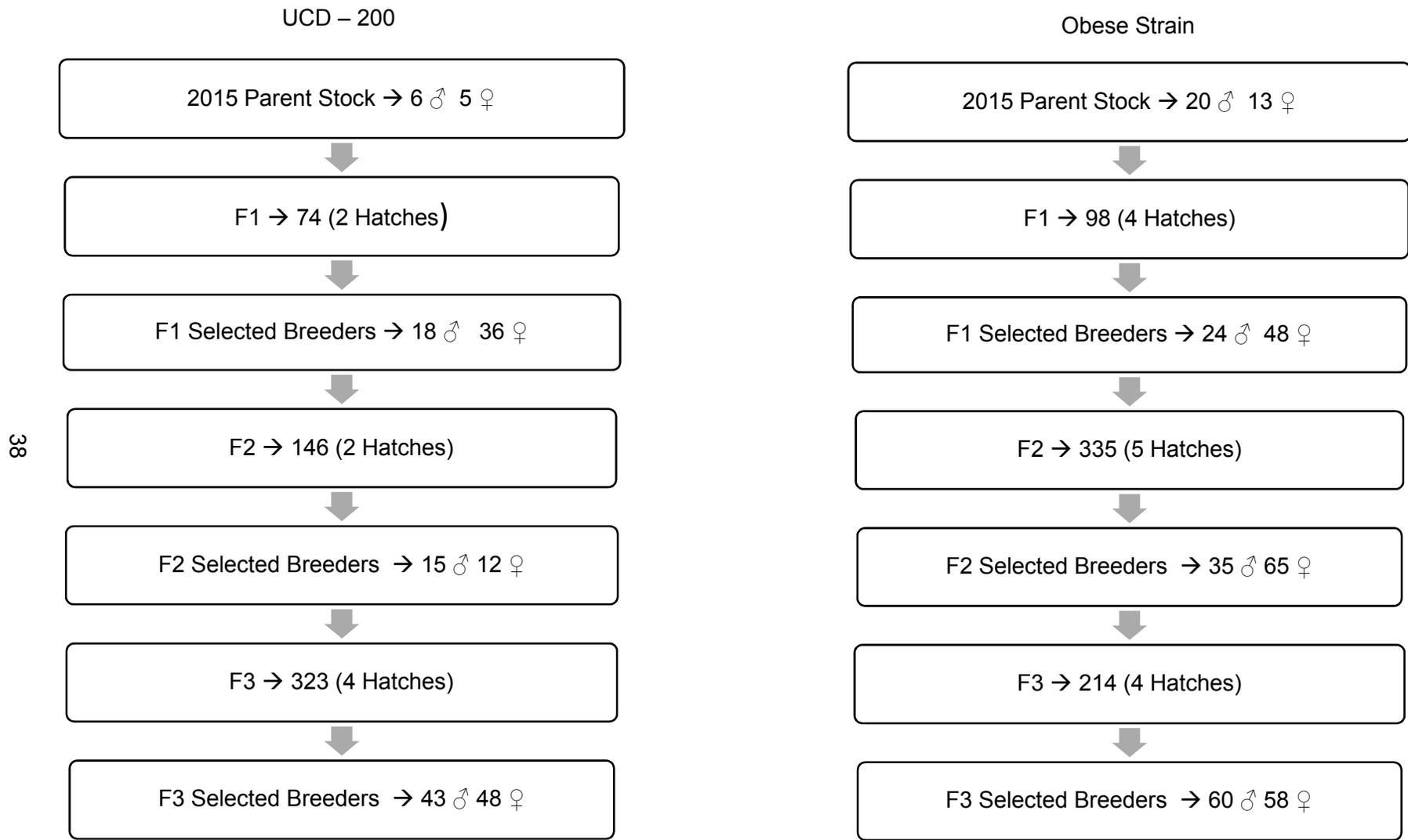
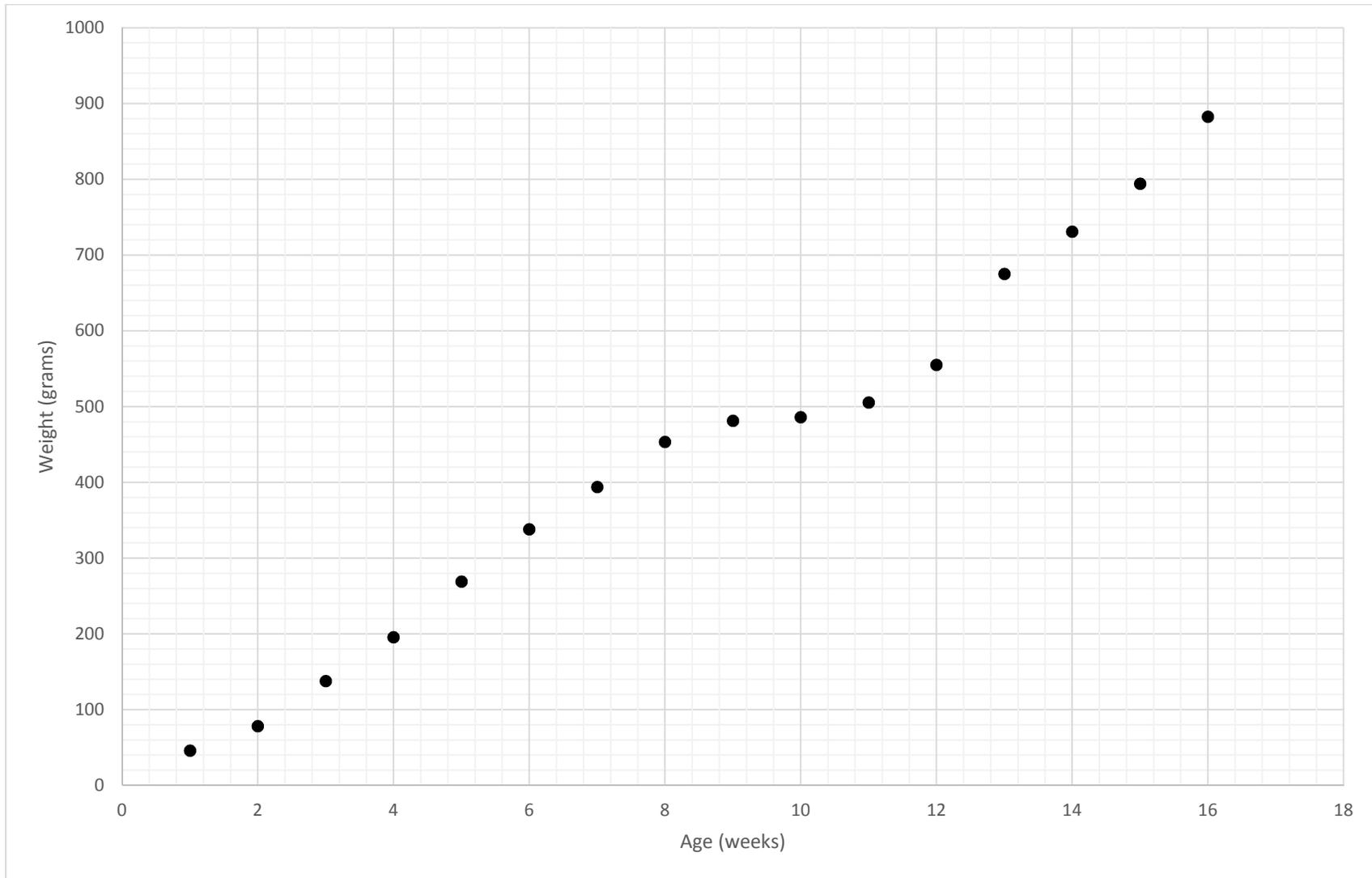
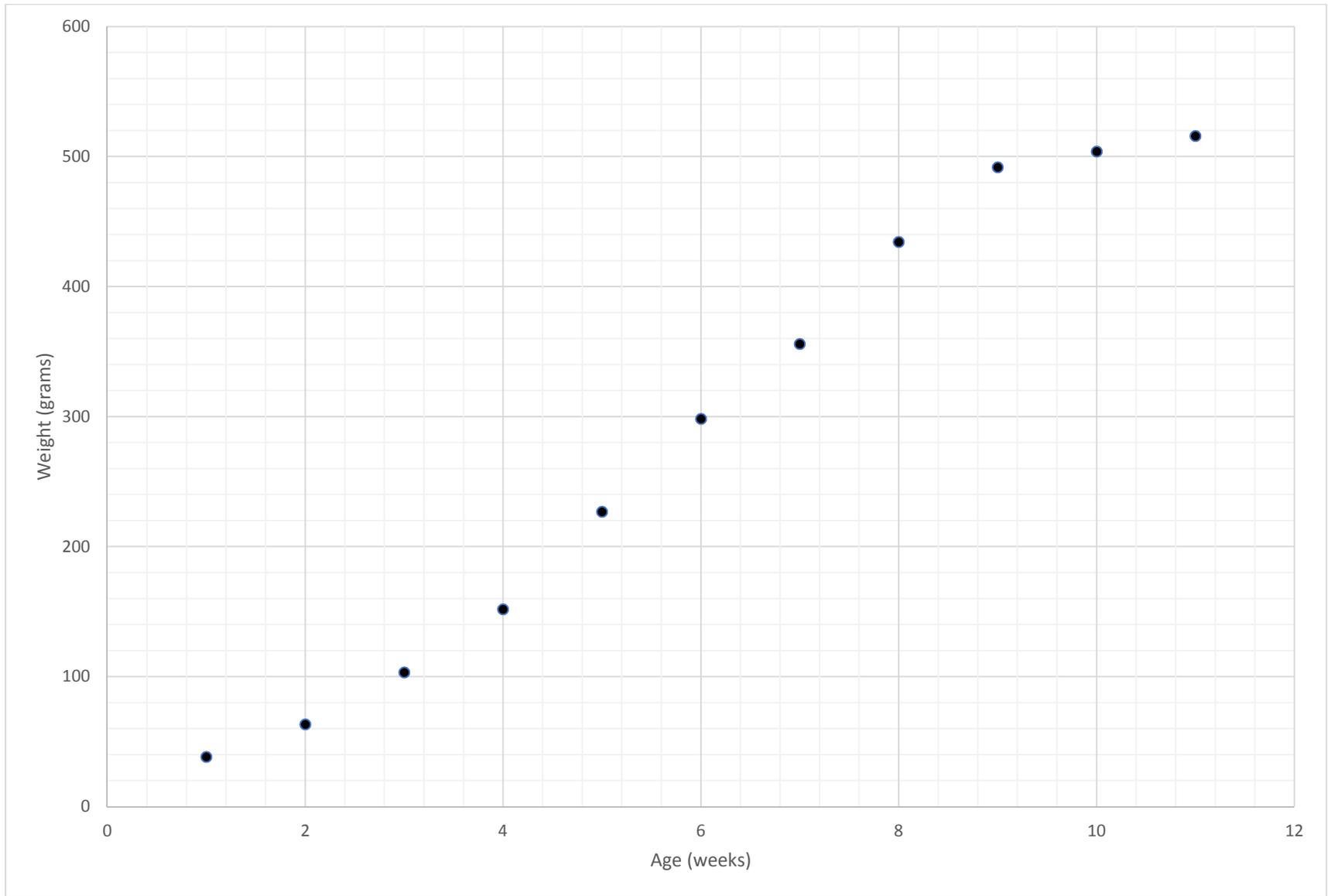


Figure 1. Rescue timeline of UCD-200 and Obese strain chickens



**Figure 2.** Average body weights of Obese Strain chickens



**Figure 3.** Average body weights of UCD-200 chickens



**Figure 4.** Dorsal Neck lesions in UCD-200 chickens (4 weeks)



**Figure 5.** Progression of comb necrosis/ “self-dubbing” phenotype

**Table 1.** MHC characterization results

UCD-200

MHC Haplotype	n	Frequency <sup>1</sup>
B2/B2	77	26.64%
B2/B15	141	48.79%
B15/B15	71	24.57%

Obese Strain

MHC Haplotype	n	Frequency
B13/13	185	100%

43

<sup>1</sup> Observed frequency of MHC type within tested populations

**Table 2.** Dorsal Neck lesion<sup>1</sup> and MHC association characterization chart

Width(mm)	n	frequency	MHC haplotype frequency <sup>2</sup> of neck lesion					
0.1-1.9	249	91.54%	B2/2	28.11%	B2/15	53.41%	B15/15	26.10%
>1.9	23	08.46%	B2/2	33.33%	B2/15	38.10%	B15/15	28.57%

<sup>1</sup> As measured using digital caliper

<sup>2</sup> Observed frequency of MHC type with respect to dorsal neck lesion width (mm)

**Table 3.** Comb necrosis characterization chart

Comb score <sup>1</sup>	n	frequency	MHC haplotype frequency of comb score					
			B2/2	27.08%	B2/15	35.42%	B15/15	31.25%
0	48	17.65%	B2/2	24.10%	B2/15	54.21%	B15/15	21.69%
1	141	51.84%	B2/2	27.33%	B2/15	49.07%	B15/15	23.60%
2	83	30.51%	B2/2	27.33%	B2/15	49.07%	B15/15	23.60%

<sup>1</sup> Scored using subjective score system; 0 indicated a normal healthy comb, 1 indicated a partial necrosis, a score of 2 indicated majority/full necrosis of the comb

<sup>2</sup> Observed frequency of MHC type with respect to comb score



To: Nicholas Anthony  
Fr: Craig Coon  
Date: February 9th, 2018  
Subject: IACUC Approval  
Expiration Date: February 1st, 2021

The Institutional Animal Care and Use Committee (IACUC) has APPROVED your protocol # **18083**: *General Rearing of Selected chicken and Quail Populations*.

In granting its approval, the IACUC has approved only the information provided. Should there be any further changes to the protocol during the research, please notify the IACUC in writing (via the Modification form) prior to initiating the changes. If the study period is expected to extend beyond February 1st, 2021 you must submit a newly drafted protocol prior to that date to avoid any interruption. By policy the IACUC cannot approve a study for more than 3 years at a time.

The following individuals are approved to work on this study: Nicholas Anthony, Sara Orłowski, and Joseph Hiltz. Please submit personnel additions to this protocol via the modification form prior to their start of work.

The IACUC appreciates your cooperation in complying with University and Federal guidelines involving animal subjects.

CNC/tmp

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