

Use of Functional Genomics to Gain New Knowledge about the Effects of Heat Stress on Mammary and Reproductive Cells in Dairy Cattle.

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Introduction

Dairy and livestock producers try to provide comfortable environments for their animals but stress can still occur. Stress comes in various ways. Heat stress is one form of stress that has been researched. Environmental heat stress on cattle can affect reproductive efficiency, milk composition and yield, and animal health. Producers know that stress on the cows must be minimized in order to maintain animal health and optimize performance. However, it is not completely understood why stress has such adverse effects on dairy cows. Recent research has focused on heat stress in dairy cattle using a new scientific area called functional genomics. Researchers Dr. Robert Collier at University of Arizona and Drs. Donald Spiers, Matthew Lucy, and James Spain at the University of Missouri are using functional genomics to investigate the reasons behind the negative impact of stress on dairy cows focusing on mammary cells, ovarian follicles and corpus lutea. Functional genomics allows researchers to explore particular production, physiological, and health situations at the gene level. It links changes in the expression of individual genes or sets of genes under different situations to the actual cell function such as milk production, muscle growth, or immune response, for example.

Outside circumstances, like stress, can send signals to cells to turn genes “on” or “off” and thereby change the regulation of various functions within the cells. Altered functions in many different cells caused by heat stress can lead to decreased milk production and increased susceptibility to infectious diseases, for example. Identifying genes that are up- or down-regulated during stress can help researchers understand more about how and why stress impacts normal functions and help resolve some of the challenges that producers face in minimizing stress to their animals. Knowledge from functional genomics can help locate genetic markers that are linked closely with genes for heat tolerance and provide opportunity to select animals with the desirable allele(s) for these genes which improve heat tolerance.

Impacts of Heat Stress

Summer heat stress is thought to cause annual losses of \$5-6 billion in decreased milk production and reproductive efficiency in the U.S. dairy industry. Although genetic tolerance to heat varies from cow to cow, research has shown that when environmental temperatures meet or exceed 35° C (95 ° F), most cows must begin to use additional energy to rid their bodies of the excess heat. They will sweat or pant and turn down or shut off processes in the body that produce heat. Cows decrease their feed intake, which decreases the heat produced from digestion, but also decreases the uptake of vital nutrients for milk production. Studies have shown that conception rates during the summer can be 50% lower than in the winter. Heat stress has been shown to negatively affect the immune system, making it more difficult for cows to ward off bacteria and

other pathogens. In fact, increased cases of mastitis and higher somatic cell counts are often observed in cows during the summer, not only because of the ideal temperature and environment for bacterial growth, but also perhaps because of decreased immune function during hot weather.

Cows can become acclimated to higher temperatures and develop a thermo-tolerance. This thermo-tolerance is associated with a family of proteins in all cells known as heat shock proteins (HSPs). These proteins, also called chaperones, have been used as an indicator of heat stress because expression of HSP genes and synthesis of HSP proteins is increased during thermal stress. When temperatures are elevated proteins begin to unfold (denature). This destroys their ability to function. The HSPs refold denatured proteins and thereby increase the cells' ability to cope with stress and survive. Thermo-tolerance at the cellular level is directly related to how long HSP synthesis is elevated during thermal stress. Cells that lose their ability to continue to make these proteins during a thermal stress do not survive.

Heat Stress on Mammary Cells

Dr. Collier and his team wanted to identify sets of genes that affect the mammary gland's ability to adapt to heat stress and to develop, grow, and produce milk. To do this, they employed functional genomics, a new science that involves a tool called a microarray. Microarrays are glass slides that are spotted with DNA (Deoxyribonucleic Acid) from thousands of genes. These slides allow researchers to look at the expression of this large number of genes at once by comparing which genes are "turned on" or "off" in cells or tissues between certain times or treatments. For example, one can look at gene expression in the liver from cows on two different types of feed, or genes in cells from diseased cows can be compared to those of healthy cows. The National Bovine Functional Genomics Consortium (NBFGC) developed a microarray that contains 18,000 genes from various bovine cells and tissues.

This particular heat stress study used epithelial cells, the cells that produce milk in the mammary gland. These isolated cells were subjected to thermal stress in the laboratory. Samples of these cells were taken to gather DNA at several time points before and after stress began. Gene expression at these time points were then compared to each other on the microarrays. For example, genes "turned on" and "off" in cells from 24 hours before any heat stress were compared to those expressed in cells that had been under heat stress for 24 hours. These epithelial cells were also viewed microscopically and physiological changes caused by heat stress are shown in Figure 1.

Analysis of changes in gene expression from microarrays determined that many genes respond to heat stress, including heat shock proteins, and cell repair genes which were up-regulated ("turned on"). Genes related to the cell cycle, metabolism, and cell structure were down-regulated. In other words, when mammary epithelial cells experience stress due to increased temperature, they shut down processes needed for growth, development, and production of milk, and speed up processes needed to survive stress. Actions in the cell that use large amounts of energy need to stop so that energy can be used to help the cell survive. These gene expression results agree with structural

changes seen in mammary cells in Figure 1. That is, cells lose their structure and ability to branch out into ducts needed for milk production. These results also help explain why milk yield is decreased in cows during hot weather. The very cells responsible for producing milk are unable to efficiently carry out functions needed to make milk. Further, it was found that when cells were exposed to heat stress for longer than 8 hours, they lost thermo-tolerance as their expression of heat shock protein fell, decreasing their chance of cell survival. At this time, expression of genes needed for programmed cell death, or apoptosis, increased, and cells began to die.

Figure 1. Microscopic view of bovine mammary epithelial cells before (a) and after (b) subjected to heat stress. Thermal stress has caused the long, branching, ductal extensions of the cell to lose their structure and ability to grow and develop to produce milk.

Figure 1a.

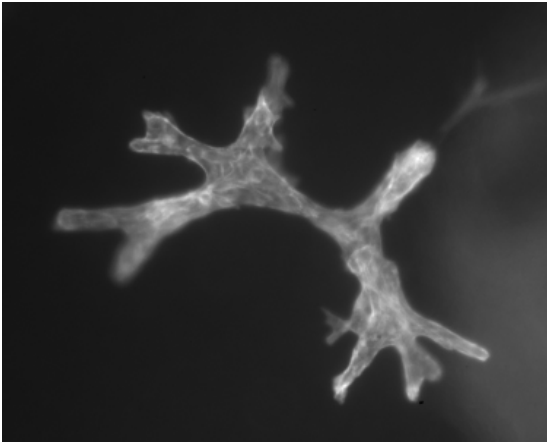
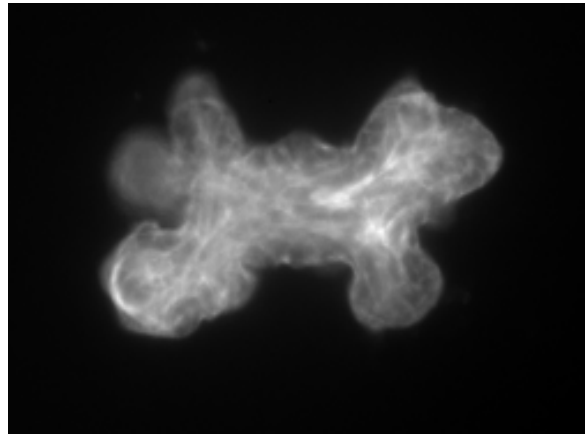


Figure 1b.



This experiment provided new knowledge about what cells are dealing with during times of heat stress. Further, this research identified potential opportunities for therapeutic treatments and genes that may be selected in dairy cows to aid in heat tolerance. In the future, Dr. Collier and his team would like to examine mammary epithelial cells from *Bos Indicus* breeds, such as Zebu and Brahman, which are better adapted to warmer climates. Perhaps these results can provide more information about why these breeds have a higher heat tolerance than production breeds in the U.S.

Impact of Heat Stress on Reproductive Cells

Dr. Donald Spiers, Dr. Matthew Lucy, and colleagues are focused on the adverse effects of heat stress on cows' reproductive efficiency. Summer conception rates can be 50% lower than winter rates. This decrease in fertility can extend into the autumn even beyond the heat stress period.

One effect of heat stress on reproduction is reduced detection of estrus. Normal behaviors seen during estrus may not be as pronounced or present at all as hot weather decreases cow movement. Producers depending on physical signs of estrus to time insemination may have lower conception rates. Bulls are physically less active and there

is a decrease in sperm quality and quantity, thereby reducing natural service conception rates.

It has been previously shown that heat stress can decrease ovarian follicular growth and reduce follicular dominance. That is, the follicle containing the egg destined to ovulate may not fully develop. And the quality and maturity of the ovulating egg may not be optimal for fertilization and production of important hormones. Another situation may occur where instead of one dominant and mature follicle developing, several codominant follicles ovulate resulting in greater incidence of twinning in the summer. In addition, heat stress decreases blood flow and increases temperature in the uterus, which increases the probability of embryonic loss.

Heat stress can negatively affect another important component of fertility, the corpus luteum (CL). The CL is the structure remaining on the dominant follicle after the egg ovulates. The CL then begins producing the hormone progesterone to maintain pregnancy. Heat stress can inhibit development of the CL which affects progesterone concentrations. Even if conception occurs, the embryo can be lost without adequate progesterone. It is important to note that in hot weather, decreased feed intake and negative energy balance also add to the above factors.

What have we learned?

Dr. Lucy chose to focus his group's research on identifying changes in dominant follicles and corpus lutea of heat-stressed animals to gain a greater understanding about decreased fertility. This group of researchers employed functional genomics and microarrays to investigate changes in gene expression. In the first of two experiments, dominant ovarian follicles were collected from synchronized and heat-stressed animals. Dominant follicles were obtained on day 15 of the estrous cycle from heifers that had been in a heat stress chamber with increasing temperature for 5 days. DNA samples were isolated from these follicles and compared to a universal bovine reference sample on the NBFGC microarray. The reference sample was made up of many different bovine tissues, including ovary tissue. A total of 109 genes encompassing a wide variety of functions changed their expression in the heat-stressed heifers. Changes seen in the genes expressed (turned on or off) helped to explain why dominant follicles experience decreased growth and development under heat stress.

In the second experiment, corpus lutea were collected from cows that had not been subjected to any heat stress and cows that had been in an environmental chamber under heat stress for 24 hours or 96 hours. Again, samples from these tissues were compared to the same reference sample on the NBFGC microarray, and a wide variety of genes were found to change their expression. Expression of heat shock protein genes went up, indicating an effort by the cells to survive. Other genes involved in cell growth and development were down regulated.

One of the main cell functions that changed was apoptosis, or controlled cell death. Apoptosis is a method for cells to essentially "commit suicide." Important examples of planned cell death include damaged or mutated DNA or immune cells that

possess potent chemicals used to kill bacteria. Apoptosis is necessary to prevent damage to the body that would occur if these cells remained active or released toxic chemicals into the bloodstream. In both dominant follicles and the corpus lutea, genes controlling apoptosis seemed to decrease their expression, indicating longer cell survival. Apoptosis genes were then up-regulated in the corpus lutea after 96 hours of heat exposure, causing cell death.

Summary

Functional genomics research is providing new knowledge about the impact of heat stress on mammary, follicle and corpus luteum cells. These research efforts may yield new answers to help producers and scientists improve dairy cow production and reproduction. Using functional genomics to identify genes that are up- or down-regulated during a stressful event can lead to the identification of animals that are genetically superior for coping with stress and toward the creation of therapeutic drugs and treatments that target affected genes. Even so, managers will continue to use various strategies to reduce animals' exposure to heat stress by providing adequate ventilation and using cooling systems when possible.