Current Topics in Biotechnology
Vol. 6, 2011

Review

The application of transcriptomics in the evaluation and understanding of bovine health and nutritional status

R. A. Murphy*
Alltech, Sarney, Summerhill Road, Dunboyne, Co. Meath, Ireland

ABSTRACT

Transcriptomics-based research initiatives will enable bovine researchers and producers to ask key questions about diet and its effects on bovine health and performance. By focusing on gene expression and functional genomics, we can gain a more definitive understanding of the importance of dietary intervention in nutritional strategies. Whilst still rapidly evolving and expanding, this new scientific frontier will revolutionise our thinking about how dietary supplementation can have such dramatic and beneficial responses on whole body health. Functional genomic studies utilising real-time PCR and high-density micro-arrays are providing new ways for researchers to rapidly evaluate the effects of diet, environment and disease processes on bovine growth and performance.

KEYWORDS: bovine, nutrigenomics, transcriptomics, microarray, review

INTRODUCTION

Over the last decade, the science of nutrigenomics has emerged. This new frontier of scientific research covers a wide range of technologies, the ultimate aim of which is to elucidate the influence of diet on the genetic programming of cells and tissues.

Considering the current global issues that are impacting on bovine production, it is evident that a number of major challenges lie ahead. Issues such as the financial crisis, resource and energy strains, population growth and increasing food demand have placed a significant strain on production capacities. Additionally, the threats associated with prions, consumer demands for antibiotic and hormone-free foods and the increasing consumer desire for functional and value-added foods will require changes in our approaches to production and efficiency. To meet these demands, producers will need to reappraise their approach to animal nutrition. This will not only result in even more maximisation of genetic potential through dietary and husbandry practices but also in the exploitation and maximisation of the genetic potential at the molecular level. Molecular potential exploitation is dependent on advances in the science of nutrigenomics, the main emphasis of which is the prevention of disease by optimizing and maintaining cellular, tissue, organ and whole-body equilibrium or homeostasis. This requires not only an understanding of, but the ability to manipulate a multitude of nutrient-related interactions at the gene, protein and metabolic levels. These new disciplines and their attendant technologies will redefine animal health and nutrition in the future.

Traditionally speaking, classical genetics as applied to animal husbandry typically relied on the phenotypical appearance of an animal due to the ease with which one could judge the outcome of a breeding program. In some respects, this has successfully led to the development and exploitation of the genetic potential of a breed. However one must remember that it is the complex interaction

*rmurphy@alltech.com

between an animal's genotype, environmental factors and random variation that will ultimately determine phenotype. With respect to environmental influences, animal nutrition has played a pivotal role in further exploiting genetic potential. Dietary intervention to prevent disease through feed supplementation has become the norm, with producers realising that not only does an animal's health and whole body homeostasis affect its performance but that maternal health can dramatically influence the performance and health of its offspring.

Bovine genomics

Ruminants possess the remarkable ability to convert fibrous material such as plant carbohydrates into volatile fatty acids (VFA) namely acetate, propionate, butyrate, lactate and valerate. Ultimately these are used as a primary energy source through fermentation by a complex community of symbiotic microorganisms present in the rumen which additionally provide the main source of dietary protein and B vitamins. Due to the unique biology of ruminants, their importance as a major source of protein nutrition for humans, and the evolutionary position of cattle relative to other mammals, the Bovine Genome Project was initiated in 2003. Through an international effort this resulted in the release of the first draft of the complete bovine genome sequence in 2004. The genome of a female Hereford cow was fully sequenced in 2009 [1]. The size of this genome is 3 Gb and is composed of 29 autosomes and two sex chromosomes [36]. Genetic information is additionally carried in a mitochondrial genome that contains roughly 16,000 base pairs. The bovine genome is roughly the same size as that of the human, containing approximately 22,000 genes of which 14,000 are common to all mammalian species and is also similar in size to other mammalian genomes. Mapping strategies have identified many complimentary markers that have helped define the structural nature of the genome, and sequencing studies have identified and cataloged well over 300,000 expressed sequence tags (ESTs) in GenBank.

In 2009, a genome-wide Survey of SNP variation was carried out by comparison of a deep draft sequence assembly of shotgun reads from a single Hereford female with sequences sampled from six additional breeds [2]. These were additionally used to develop probes to interrogate 37,470 single-nucleotide polymorphisms (SNPs) in 497 cattle from 19 phenotypically and genotypically diverse breeds. The results of such analysis indicate that cattle have undergone a decrease in effective population size from a large ancestral population. This is thought likely to be due to factors such as domestication, selection, and breed formation which have left detectable signatures within the cattle genome. The mapping of key DNA differences, (haplotypes), between several varieties of cattle could allow us to understand the role of genes coding for products of economic value such as milk and meat. In addition it opens opportunities for enhancing selective breeding and changing certain cattle characteristics. The evolutionary relationships between bovine and mammalian species will enable a comparison between genomic similarities and differences and also allow for comment on mammalian biology. Of particular interest are the molecular bases for economically important traits in ruminants such as lactation, metabolism, reproduction and disease resistance. These are important scientific issues which through comparative analysis of the cattle genome with other mammalian genomes can be addressed.

Utilisation of genomic information

Studies of the basic biochemistry behind genetics, the genetic structure, and the basic flow of information in biological systems have fostered the development of a multitude of new genomic-associated disciplines. These are generally centered around key molecular methodologies that were developed to increase our knowledge of the basic molecular structure of life. The sciences that make up functional genomics include transcriptomics, proteomics, and metabolomics, which study the quantitative relationships between the genome and gene expression, protein production, and metabolic processes, respectively. At its very basic level, biology can be defined by a central dogma that describes flow of biochemical information from DNA to RNA and then to protein. As a result, the information contained in the nucleotide base sequence in DNA determines

the basic amino acid sequences in protein and plays a large role in determining the structural and functional nature of the encoded proteins.

All biological processes are dependent on the regulation or control of the information flow in this pathway. While this process is carefully controlled by the principal genetic determinants, many external factors can also influence its regulation. Such factors include disease challenge, exposure to environmental toxins, and nutrient supply. The basic understanding of these complex regulatory processes has changed considerably with the delineation of the various animal, plant, and microbial genomes. It is now possible to understand these regulatory processes in extremely fine detail. One step in this pathway, the transcription of a gene sequence into mRNA, is currently being described by examining factors that influence the expression of specific genes and the transcription of their corresponding mRNA. The science of transcriptomics is based on the examination of gene expression patterns resulting from quantitative examination of the abundance of mRNA copied from a nucleic acid blueprint contained in the genome.

Transcriptomics and the use of microarrays for evaluating gene expression

In the last 10 years, our knowledge of nucleic acid sequences, nucleic acid hybridization, and cloning techniques has provided tools that can be used to gain a clearer understanding of overall gene expression at the transcriptional level. While techniques to study the expression of individual genes have been available for many years, oligonucleotide and cDNA microarrays have provided powerful tools that will allow rapid evaluation of gene expression on an unprecedented scale. These techniques are based on a quantitative assay of the relative concentrations of specific RNA messengers (mRNA) in cells and tissue samples. The relative amount of individual mRNA molecules present in a given tissue or cell directly reflects the level of gene regulation and can be used to quantitatively examine the factors that regulate the gene expression. The amount of the mRNA transcript present in tissues can be measured indirectly after it is extracted and then used to create a complimentary labeled strand of DNA. This labeled material can be hybridized with a complimentary strand on an array containing a known set of gene sequences that are attached to a solid glass slide or nylon substrate. The sequences are often organized as an array of small spots on the solid matrix and are generally referred to as probes. The intensity of the color that results during the hybridization process is directly related to the amount of target mRNA present and reflects the level of gene expression. In this way, it is possible to determine which gene is up-regulated or down-regulated as a result of specific biological manipulations or during normal tissue development. Comparison studies of gene regulation can be carried out using subtractive hybridization procedures that use contrasting color labels on complimentary DNA from two sets of messengers from different tissues [3]. As a result, it is possible to quantitatively compare gene expression in two contrasting groups of tissue or animals. By using robotic techniques for producing arrays on a minute scale and laser techniques to discern the color of specific spots, it is possible to examine the expression of thousands of genes at one time. This is an extremely powerful tool that can be used to study metabolic processes at a very fundamental level and lends itself well to the complex understanding of interactions that regulate gene function.

Since gene transcription is only one step in the regulatory pathway that leads to functional protein formation, it is not always possible to correlate the increased presence of mRNA in the tissue with phenotypic or protein changes in tissues [3, 4].

While studies of gene transcription may have many drawbacks in this respect, the ability to globally evaluate the initial regulatory steps in gene expression provides many tools for elucidating the key processes in metabolic regulation. Powerful screening methods are now available to identify the key gene expression patterns that are influenced by environment, disease, and nutrition or simply during the process of tissue development.

In the past, microarray studies have depended on specific arrays with relatively few nucleotides and limited amounts of information. These arrays were often generated to examine specific metabolic

functions or immune responses. Recent work has reported the development of arrays that can be used to examine gene expression in a variety of species. These arrays range in size from a few hundred probes to systems that have over 40,000 elements. While the use of smaller, more defined arrays to examine regulation of specific tissue response have been useful, the development of standardized systems for examining the expression of large numbers of genes will greatly enhance our ability to understand basic metabolic and physiological functions.

Current applications of transcriptomics in bovine health and nutrition

Transcriptional profiling of gene expression has the potential to provide profound new insights into ruminant physiology, health and nutrition. Practically speaking, gene expression studies will allow for the identification of pathways and candidate genes responsible for economically important traits through the use of bovine specific microarrays that are specifically designed for bovine studies.

At this point, many of the more complex arrays for ruminants are based on expressed sequence tags (ESTs) that have not been associated with specific genes or gene functions [5].

While these arrays can be used to compare and differentiate gene expression patterns, the lack of well annotated probes limits their immediate usefulness in identifying specific functional changes or physiological processes. Numerous bovine-specific arrays have been developed and have been used to look at changes in gene expression in bovine tissue [5]. However, the release of a more advanced high-density bovine microarray with the ability to monitor the expression of approximately 23,000 transcripts promises to help standardize gene expression studies in the future (GeneChip® Bovine Genome Array, Affymetrix, Santa Clara, CA). Assessment of the biochemical and physiological effects of these genes and their gene products is still limited by the slow progress in annotating the ESTs.

While largely of a descriptive nature, global approaches to transcriptomic-based evaluations in gene expression and regulation in cattle have been utilised to evaluate the effects of immunological challenge and disease processes [6, 7, 8, 9, 10].

Several studies utilising microarrays in cattle have focused on characterization of gene expression during the development of embryos, during pregnancy, and during the periparturient periods and provide insights into fertility processes [11, 12, 13, 14, 15]. Although basic and illustrative, these approaches have begun to catalog the gene expression changes associated with embryo implantation and development.

Initial studies of gene expression during the development of the bovine embryo have suggested a number of candidate biomarkers that can be used to follow the key changes in the embryo during development [14]. These gene expression studies may provide clues about important physiological and developmental processes, however no universal gene markers that can be specifically associated with fertility or embryo survival have been identified.

These early studies were limited by a lack of functional information, however the full sequencing of the genome has allowed for much more advanced and sophisticated analyses to be carried out. For instance Southey *et al.* [16] used complementary bioinformatic resources including genomic libraries and cleavage prediction approaches to identify 92 cattle prohormone genes, with 84 of those being supported by expressed sequence tags. Notable findings from this study included an absence of evidence for a cattle relaxin 1 gene and evidence for a cattle galanin-like peptide pseudogene.

Ekman *et al.* [17] presented the analysis of the immunoglobulin and surrogate light chain gene assortment extracted from the *Bos taurus* genome sequence Btau_3.1. This notable paper detailed some key findings from which an interspecies comparison suggested ruminant specific adaptations including the preferential use of the λ light chain in cattle. It also suggested that in cattle, the number of functional immunoglobulin light chain genes is remarkably lower than in mice and in man.

The completion of the bovine genome has allowed for the microarray analysis detailed by Rodriguez-Osorio *et al.* [18] which explored the transcriptome

profile of cloned embryos relative to that of the donor cells and IVF embryos as a control. Given that *in-vitro* culture conditions may alter gene expression and may lead to developmental aberrations in IVF derived cattle, this work is important in that it provides a data set that could contribute to our understanding of epigenetic errors in somatic cell chromatin transfer and have implications for both basic and applied research.

Walker and Roberts [19], characterised the bovine type I IFN locus by annotating data from the recently completed genome and showed significant gene rearrangements, expansions, and novel subfamilies when compared to other mammalian models. These insights into the functional evolution of the type I IFN in ruminants indicate that the species may have required an expansion of the Type I IFN locus during its evolution. The authors postulate that this may have been required to provide the broader anti-viral protection required for foraging and foregut fermentation. On a practical level this type of information may provide for the identification of targets for anti-viral development.

A comparative analysis of the gene expression profile of B. abortus-infected monocyte-derived macrophages (MDMs) from naïve cattle naturally resistant (R) or susceptible (S) to brucellosis using a cDNA microarray technology has also been detailed [37]. This study yielded some unique insights into host response to infection and may lead to the development of strategies whereby the host response can be manipulated to not only control but perhaps prevent infection by brucellae.

A key question in biology concerns the clustering of genes with respect to their known functions or phenotypic effects. This is of particular interest for QTL's where a trait conferring region could contain a number of genes that contribute to the trait being measured. Salih and Adelson, [20] carried out an analysis of the QTL vs non-QTL (no QTL coverage) regions of the bovine genome. The authors provided evidence that gene density is higher in QTL regions and that some gene functions were also over-represented in QTL regions. While many of these were not obviously linked through a biological context, the results were consistent with the hypothesis that genes may be clustered in a manner that reflects their functional association with particular traits. The authors showed that this was most obvious for the 'milk yield' QTL category, where the associations noted were considered as biologically plausible.

Obviously the linkage of QTL data through genomic studies will allow for an understanding of the complex and interconnected molecular pathways controlling cellular and molecular biochemistry. This type of research could allow for a more targeted approach to selection and breeding programs.

Timperio *et al*. [21] utilized a transcriptomics-based approach to assess the differential expression of gene profiles from two separate bovine breeds - Holstein-Friesian and Chianina. The use of proteomics data allowed for an integrated approach to assess physiological differences in the liver function of the animals. The authors noted that despite being closely related at the genetic level, a disparity was noted between the proteomic and transcriptomic profiles. Approaches such as this integrated study provide molecular tools enabling an examination of the physiological differences between Holstein and Chianina cattle breeds and ultimately will contributes to a more detailed understanding of the important biological processes which have resulted in the development of distinct breeds.

A recent study [22], illustrated the potential for using transcriptomics to detect the elicit use of anabolic agents.The authors assessed both post-pubertal Nguni heifers and pre-pubertal Holstein-Friesian calves. Application of such an approach demonstrated its potential application in establishing gene expression patterns which could be used as biomarkers for illegal application of exogenous hormones. Interestingly, the authors demonstrated that this approach would only be valid in post-pubertal heifers.

Challenges associated with the use of transcriptomics

While a number of studies have begun to examine the complex genomics and transcriptomics of *Bos Taurus*, these studies only serve to re-emphasize the complexity of gene expression patterns in mammalian species and indicate the need for long term research aimed at bridging the disconnect

that currently exists between understanding the relationship between genotype and phenotype.

Relaxin, a polypeptide hormone normally associated with pregnancy is a good example of this disconnect. As a hormone it has a role in reproductive tract metabolism, connective tissue remodeling, and myometrial contractility, although more recently a functional gene has been shown to be absent from the bovine genome [16]. Cross species functionality has however been demonstrated and the absence of a bovine relaxin gene is interesting given that previous research has demonstrated that cows injected with porcine relaxin produce a classical response in which an increase in the rate of pelvic area expansion and a dilation of the cervical sphincter are noted [23]. Additional work [24] showed that porcine relaxin in combination with dexamethasone and cloprostenol could reduce the incidence of retained placenta in beef heifers. Given that a relaxin-type physiological response can be elicited despite the absence of a relaxin gene homologue, we can speculate that at the very least, functional receptors for this hormone exist and remain to be found or perhaps that as yet an unidentified gene or pseudogene encoding a relaxin type function exists.

The possibility also remains that the bovine variant may be too evolutionarily distant to be identified by probes based on other species. For instance the ovine relaxin-like pseudogene is characterized by an mRNA transcript containing multiple stop codons in all possible reading frames. A translation of this mRNA to protein would produce a vastly truncated, non-functional peptide [25]. Examining the evolution of ruminants and also of relaxin it is of interest that camels express a relaxin gene, sheep express a non-functional gene product and cattle appear devoid of such. In phylogenetic terms, sheep have been shown to have evolved after the camel, but before cattle which potentially suggests the presence of a pseudogene, redundant cellular activities or the complete absence of a bovine relaxin variant.

In evolutionary terms it would appear that the physiology of relaxin mediated biological response has either been lost in the gradual transition noted with bovine evolution or has progressed beyond the current knowledge and understanding of this hormone. Clearly, the use of transcriptomics- based cross-species studies may allow researchers to bridge some of the disconnect that exists in understanding the precise relationship between genotype and phenotype.

As an example, to fully appreciate the benefits of nutritional supplementation, genotypic changes, which are in part responsible for the observed physical changes, must be considered. Dawson and co-workers initiated a nutrigenomics-based research programme to study the effects of organic selenium supplementation at the molecular level [5]. Using microarray technologies, a specific branch of nutrigenomics, transcriptomics, was utilised to determine how nutritional exposure to organic selenium affects gene expression in a tissue-specific fashion. The results of this work have reinforced our thinking on the health benefits to be derived from selenium addition to the diet. However, it should be noted that it is only through applying a systems biology approach to the interpretation of experimental data derived from transcriptomics work, that we will be able to fully understand and explain the health benefits of dietary intervention and supplementation. This is an area that is currently being pursued to enable a full understanding of the transcriptomics results that have been derived from model-animal studies.

Work carried out by this group using a poultry model system may allow us to speculate that the micro-nutrient selenium is capable of eliciting a relaxin-type physiological response through an as yet unidentified pathway. This speculation is based on the positive stimulation of relaxin gene expression following administration of a selenium enriched yeast to poultry diets [5], and the numerous publications demonstrating that selenium can reduce the incidence of retained placenta in both bovine and equine species [26, 27]. Although speculative, this type of nutrigenomics-based approach may allow researchers to identify previously hidden effects of nutrition on health and performance. In this instance the conundrum surrounding the precise physiological role of selenium in reducing retained placentas in ruminant species may be elucidated through the use of transcriptomics- based studies.

The importance of the micro-nutrient selenium in this case may lie in its ability to stimulate relaxin type responses through evolutionarily redundant pathways. Obviously more research is required to fully elucidate the mechanism by which this occurs.

The ruminant as a model system

As a non-human mammal, the ruminant genome is ideally placed for extensive study to understand mammalian physiology. Ultimately the expectation is that discoveries made in the ruminant may perhaps provide insights into the genomic architecture of other mammalian species. In particular the potential to explore the causes and treatments for human disease is of interest, a good example of which is the shared commonality of tuberculosis. Whilst studying model organisms can be informative, care must be taken when generalizing from one organism to another.

The ruminant immune system for instance has undergone an evolutionary adaptation to the extensive microbial communities that populate its gut and exposed epithelial surfaces. A good example of this can be found in the peripheral blood lymphocytes in which 40% have γ/δ T-cell receptors as compared to less than 1% in humans and rodents. As a consequence we can consider the ruminant as an excellent model species to study the evolution and function of these T-cells which play a role in innate and adaptive immunity processes. Ekman *et al*. [17] extracted the bovine immunoglobulin lambda (λ) chain locus from which the data on the immunoglobulin light chain genes provides novel insight on the humoral immune system of ruminants. This should facilitate the development of vaccines and other therapeutic tools against cattle specific infectious diseases.

Notably when compared with humans and rodents, significant changes have been noted in the organization of several immune gene families in cattle [28]. Examples of these can be observed with the arrangement of the serum amyloid A3 [29] and type 1 interferon [19] families, as well as the major histocompatability complex [30] and T cell receptor B locus [31]. These genomic rearrangements suggest that there has been a differential adaptation to infectious disease challenges [28].

An analysis of the genes encoding bovine proteins and the genes expressed in mammary tissue during the lactation cycle has provided the first comprehensive overview of lactation genomics. This information will ultimately provide a resource for the identification of genes involved in complex dairy production traits. These analyses have also demonstrated that milk is not simply a source of high quality nutrients but it also contains a variety of bioactive proteins which presumably play key roles in the antimicrobial defense system of the newborn calf and possibly also in gut maturation [32]. Ultimately this type of research focus may identify directions of research for additional mammalian species, in particular humans.

Additionally, alterations in the organization of gene families involved in reproduction have been observed which when compared with the other mammalian species are reflective of the distinctive nature of reproduction in cattle, particularly with regard to differences in placental structure. From this work a number of genes were implicated in various aspects of bovine reproductive function and early development [18, 33].

Gene losses in cattle when compared with their retention in other mammalian species may be important for understanding the metabolic adaptation to the ruminant lifestyle [34, 35].

Bovine research beyond the genome

Following on from the completion of the bovine genome sequence, the main challenge facing bovine scientists is the utilization of this information to improve all facets of poultry production. The coordination of existing resources such as genomic and phylogenetic data, quantitative trait loci (QTL) markers, expressed sequence tag (EST) libraries, the ever improving microarrays, and the so called ‘omics’ tool sets will allow for an understanding of the complex and interconnected molecular pathways controlling cellular and molecular biochemistry. The genome sequence has already begun to facilitate the study of genes and their regulatory elements, the subsequent gene products and the gene expression patterns for various metabolic processes. The bovine genome will be essential to predict the

amino acid sequences of encoded proteins/ peptides, thus facilitating the development and utilization of proteomics-based research approaches. Ultimately, the completed bovine sequence has allowed for a rapid and simplified tool allowing scientists to search for candidate genes that are in close proximity to a marker linked to a desirable trait thus accelerating and increasing the breeding potential.

Inevitably the bovine genome sequence and additional haplotype mapping will enable a revolution in the beef and dairy industries through providing a means to address key livestock issues namely efficient and sustainable production systems and the reduction in environmental footprints.

REFERENCES

1. Bovine Genome Sequencing and Analysis Consortium, 2009, Science, 324, 522-528.
2. Bovine HapMap Consortium Genome, 2009, Science, 324, 528-532.
3. Moody, D. E. 2001, J. Ani. Sci., 79(Suppl. E), E128-E135.
4. Muller, M. and Kersten, S. 2003, Nature Rev., 4, 315-322.
5. Dawson, K. A. 2006, Biotechnology in the Feed Industry, Proceedings of Alltech's 22nd Annual Symposium, Lyons, T. P. and Jacques, K. A. (Eds.) Nottingham University Press, Nottingham, UK.
6. Burton, J. L., Madsen, S. A., Yao, J., Sipkovsky, S. S., and Coussens, P. M. 2001, Acta Vet. Scand., 42, 407-424.
7. Tao, W., Mallard, B., Karrow, N., and Bridle, B. 2004, Vet. Immunol. Immunopathol., 101, 117.
8. Donaldson, L., Vuocolo, T., Gray, C., Strandberg, Y., Reverter, A., McWilliam, S., Wang, Y. H., Byrne, K., and Tellam, R. 2005, BMC Genomics, 6, 135.
9. Chitko-McKown, C. G., Fox, J. M., Miller, L. C., Heaton, M. P., Bono, J. L., Keen, J. E., Grosse, W. M., and Laegreid, W. W. 2004, Dev. Comp. Immunol., 28, 635-645.
10. Marsh, I. B., Bannantine, J. P., Paustian, M. L., Tizard, M. L., Kapur, V., and Whittington, R. J., 2006, J. Bacterial., 188, 2290-2293.
11. Ishiwata, H., Katsuma, S., Kizaki, K., Patel, O. V., Nakano, H., Takahashi, T., Imai, K., Hirasawa, A., Shiojima, S., Ikawa, H., Suzuki, Y., Tsujimoto, G., Izaike, Y., Todoroki, J., and Hashizume, K. 2003, Mol. Reprod. Dev., 65, 8-18.
12. Yao, J., Ren, X., Ireland, J. J., Coussens, P. M., Smith, T. P. L., and Smith, G. W. 2004, Physiol. Genomics, 19, 84-92.
13. Madsen, S. A., Chang, L. C., Hickey, M. C., Rosa, G. J. M., Coussens, P. M., and Burton, J. L. 2004, Genomics, 16, 212-221.
14. Ushizawa, K., Herath, C. B., Kaneyama, K., Shiojima, S., Hirasawa, A., Takahashi, T., Imai, K., Ochiai, K., Tokunaga, T., Tsunoda, Y., Tsujimoto, G., and Hashizume, K. 2004, Reprod. Biol. Endocrinol., 2, 77.
15. Herath, C. B., Shiojima, S., Ishiwata, H., Katsuma, S., Kadowaki, T., Ushizawa, K., Imai, K., Takahashi, T., Hirasawa, A., Tsujimoto, G., and Hashizume, K. 2004, BBRC, 313, 666-680.
16. Southey B. R., Rodriguez-Zas, S. L., and Sweedler, J. V. 2009, BMC Genomics, 10, 228.
17. Ekman, A., Niku, M., Liljavirta, J., and Iivanainen, A. 2009, BMC Immunology, 10, 22.
18. Rodriguez-Osorio, N., Wang, Z., Kasinathan, P., Page, G. P., Robl, J. M., and Memili, E. 2009, BMC Genomics, 10, 190.
19. Walker, A. and Roberts R. M. 2009, BMC Genomics, 10, 187.
20. Salih, H. and Adelson, D. L. 2009, BMC Genomics, 10, 184.
21. Timperio, A. M., D'Alessandro, A., Pariset, L., D'Amici, G. M., Valentini, A., and Zolla, L. 2009, J. Proteom., 73, 309-322.
22. Becker, C., Riedmaier, I., Reiter, M., Tichopad, A., Groot, M. J., Stolker, A. A. M., Pfaffl, M. W., Nielen, M. F. W., and Meyer, H. H. D. 2011, J. Steroid Biochem., 125, 192-201.
23. Perezgrovas R. and Anderson L. L. 1982, Biol. Reprod., 26, 765-776.
24. Musah, A. I., Schwabe, C., Willham, R. L., and Anderson, L. L. 1988, Anim. Reprod. Sci., 16(3-4), 237-248.
25. Roche, P. J., Crawford, R. J., and Tregear, G. W. 1993, Mol. Cell Endocrinol., 91, 21-28.

26. Julien, W. E., Conrad, H. R., and Moxon, A. L. 1976, J. Dairy Sci., 59(11), 1960-2.
27. Kommisrud, E., Østerås, O., and Vatn, T. 2005, Acta Vet. Scand., 46, 229-240.
28. Tellam, R. L., Lemay, D. G., Van Tassell, C. P., Lewin, H. A., Worley, K. C., and Elsik, C. G. 2009, BMC Genomics, 10, 193.
29. Molenaar, A. J., Harris, D. P., Rajan, G. H., Pearson, M. L., Callaghan, M. R., Sommer, L., Farr, V. C., Oden, K. E., Miles, M. C., Petrova, R. S., Good, L. L., Singh, K., McLaren, R. D., Prosser, C. G., Kim, K. S., Wieliczko, R. J., Dines, M. H., Johannessen, K. M., Grigor, M. R., Davis, S. R., and Stelwagen, K. 2009, Biomarkers, 14, 26-37.
30. Brinkmeyer-Langford, C., Childers, C. P., Fritz, K., Gustafson-Seabury, A., Cothran, M., Raudsepp, T., Womack, J. E., and Skow, L. C. 2009, BMC Genomics, 10, 182.
31. Connelley, T., Aerts, J., Law, A., and Morrison, W. I. 2009, BMC Genomics, 10, 192.
32. Lemay, D. G., Lynn, D. J., Martin, W. F., Neville, M. C., Casey, T. M., Rincon, G., Kriventseva, E. V., Barris, W. C., Hinrichs, A. S., Molenaar, A. J., Pollard, K. S., Maqbool, N. J., Singh, K., Murney, R., Zdobnov, E. M., Tellam, R. L., Medrano, J. F., German, J. B., and Rijnkels, M. 2009, Genome Biol., 10, R43.
33. Ulzun, N., Rodriguez-Osorio, A., Kaya, H., Wang, J. J., Parrish, V. A., Ilyin, E., Memili, E. 2009, BMC Genomics, 10, 183.
34. Seo, S. and Lewin, H. A. 2009, BMC Sys. Biol., 3, 33.
35. Weikard, R., Goldammer, T., Eberlein, A., and Kühn, C. 2009, BMC Genomics, 10, 186.
36. Bovine Genome Sequencing and Analysis Consortium, 2009, Science, 324, 5926.
37. Rossetti, C. A., Galindo, C. L., Everts, R. E., Lewin, H. A., Garner, H. R., and Adams, L. G. 2011, Res. Vet. Sci., 91(1), 40-51.