Anim Reprod, v.9, n.3, p.217-222, Jul./Sept. 2012

Research in animal reproduction: Quo vadimus?

B.D. Murphy

Centre de Recherche en Reproduction Animale, Faculté de Médecine Vétérinaire,
Université de Montréal, St-Hyacinthe, QC, Canada.

Abstract

Population growth and trends in food consumption are expected to result in a net food deficit and widespread loss of food security across the globe within four decades. It is generally accepted that this crisis will have to be met by increased livestock production, using less land, less water and in an environmentally sustainable fashion. As animal reproduction and reproductive efficiency are the basis of livestock production, it is essential that technological advances be made to increase the animal-based food supply. Improvements are required in artificial insemination procedures, in embryo transfer and in transgenic animal production. Technology is evolving such that it may soon be possible to rapidly sequence genomes and transcriptomes to hasten genetic improvements, to produce gametes from stem cells, and to increase success rates in livestock transgenesis. The principal constraints at this time are on research funding and on the paucity of scientists with multidisciplinary skills. Given its livestock population, its biodiversity, and its burgeoning scientific expertise, Brazil is expected to be a major contributor to the resolution of food security problems in coming years.

Keywords: embryo transfer, genome sequencing, reproduction, technology.

The coming crisis

The current world population is more than 7.1 billion people. Reliable estimates indicate that more than 1 billion, or some 14% of the current population, live under conditions of insufficient food supply (Foley *et al.*, 2011). Indeed, some 15 million children die from famine annually. Population growth is expected to continue, with an estimated increase to 9.1 billion by 2050. No regions in the world are immune to loss of food security. According to recent reports from the Food and Agriculture Organization of the United Nations, livestock contribute 40% of the total value of worldwide agricultural output. As countries and regions move toward becoming more industrially developed, the preferences and requirements for livestock-derived components in the diet (eggs, meat and milk) increase. Over the last 40 yr, the worldwide consumption per capita of milk has doubled, while meat consumption has more than tripled (Kearney, 2010). The augmentation in meat consumption has been much higher in some regions, with nine-fold increases in China (Kearney, 2010). Major changes are needed in agricultural practices, in developing food supplies and in managing the consumption of foodstuffs to meet the current and coming needs of the human race. It is estimated that food production will need to double by 2050 to address the current deficit and population increase (Foley, 2011).

Recent studies indicate that livestock production contributes to ecosystem destruction through several mechanisms, including large-scale monoculture of grassland species for animal feed (Balmford *et al.*, 2012) and pollution of surface waters by fertilizer and animal wastes (Place and Mitloehner, 2010). Further, animal agriculture contributes substantially to the deterioration in air quality and to global warming by producing gases with greenhouse effects. It is estimated that the agricultural sector, and primarily livestock production, contributes significantly to climate change (Place and Mitloehner, 2010). In addition, dairy cows and dairy cow manure produce volatile organic compounds and carbon dioxide, methane and nitrous oxide, all contributing to global warming (Godbout *et al.*, 2010). Indeed, methane and nitrous oxide are twenty-fold more potent than carbon dioxide in affecting climate change. The combination of methane, nitrous oxide and carbon dioxide from animal agriculture has been estimated to account for 18% of global greenhouse gas emissions (Friel *et al.*, 2009).

Demographic shifts and changes in land use dictate the viability and practice of animal agriculture, and the importance of these considerations will increase in future years. In developed countries, there is a definitive trend away from smallholder operations, toward larger and more efficient settings for animal production. The amount of land and water resources available for livestock production is projected to decline considerably over the next decades. The growing trend toward use of agricultural plant production to produce biofuels to satisfy energy needs is expected to have an impact on the cost and availability of feed for livestock (McMichael *et al.*, 2007). Increased value of crop production also encourages use of pasture for raising biofuel crops, thereby further reducing the available feed resources for animal agriculture. Nonetheless, ruminants, due to their capacity to ferment plant material and utilize cellulose, can thrive on forage land not suitable for crop farming, conferring an advantage to animal production.

In overview, animal agriculture of the future

[1]Corresponding author: bruce.d.murphy@umontreal.ca

Received: May 19, 2012

Accepted: June 29, 2012

will have to produce more meat, milk and eggs, on less land, with a potentially reduced feed supply, and in an environmentally sustainable manner. As reproduction is central to livestock husbandry, reproductive technology will be essential to meet these formidable challenges to the food supply of the 21st century.

Technology currently available

The scientific investigation that resulted in development and adoption of the first generation biotechnology of artificial insemination, beginning in the mid-twentieth century, represents one of the most valuable innovations to animal agriculture since domestication. Genetic selection and the use of artificial insemination have more than doubled lifetime milk yields per dairy cow. Beef cattle feed conversion and meat production have been improved, and currently much of the genetic improvement that can be seen in swine herds is attributable to the widespread use of artificial insemination. The major limitations to use of this procedure have been the success rates, at best about 65% in cattle. To address the major impediment, heat detection, systems for fixed-time artificial insemination have been developed. These systems have evolved from discovery of the luteolytic effects of F-series prostaglandins, and currently include use of prostaglandins to regress corpora lutea, progestagens to mimic luteal function, regulation of follicular development with estrogens or GnRH, and synchronization of ovulation with GnRH or estrogens (Bo *et al.*, 2007; Lamb *et al.*, 2010; Baruselli *et al.*, 2011). While this technology has a number of benefits, there remains substantial potential for improvement in the rate of successful pregnancy.

In the animal industry, it is often of benefit to produce offspring of one gender or another. Production of female calves in the dairy context represents the best example of this benefit. This has been addressed by technology to separate sperm by flow cytometry to inseminate with exclusively X-chromosome bearing gametes (de Graaf *et al.*, 2009). These methods have been successful in many species of domestic animals, with limitations. The separation is very slow and costly, with considerable sperm loss and the consequent product has abbreviated fertilizing life-span (de Graaf *et al.*, 2009). Other approaches may become available. In humans, fetal cell-free DNA from the syncytiotrophoblast can be found in maternal plasma early in gestation, and can be amplified by PCR to determine genetic characteristics, including gender (Avent *et al.*, 2009; Kitzman *et al.*, 2012). This approach appears not to have been employed in livestock, but may be impaired by placental differences, given that the ruminant trophoblast is not invasive, and the placentae of ruminants and pigs are the epitheliochorial type. In both cases, one would expect later occurrence and lower abundance of cell-free fetal DNA in maternal circulation. This notwithstanding, it clearly merits further exploration as a means of sampling genomic characteristics, including gender early in gestation.

Since its introduction more than 40 yr ago, embryo transfer has become routine in cattle, and the technology has been widely applied to other livestock species. Its potential for genetic gain by amplification of genomes of both sire and dam is well appreciated. It also serves as the mechanism for propagation of cloned animals and of transgenes. Limitations in the technology employed include the efficacy of superovulation (Mapletoft and Bo, 2011), the follicle reserve of the embryo donor (Sills *et al.*, 2009; Ireland *et al.*, 2011; Kitzman *et al.*, 2012) and the success of gestation in the recipient. The incorporation of technology for fixed-time artificial insemination of donors and fixed-time transfer of embryos has reduced animal handling, but there is room for improvement in conception rates (Baruselli *et al.*, 2011). Increases in efficiency will require development and implementation of technology that will result in vitro embryogenesis, improved superovulation, better synchronization of donor and recipient, and better evaluation of embryo quality.

Technology for global evaluation of the genome that is currently available will soon permit informed selection and rapid genetic gain. Single nucleotide polymorphisms (SNP) have proven highly informative in identifying human genomic differences (de Leeuw *et al.*, 2011). Bovine SNP arrays are commercially available, and are being employed in a number of contexts to explore genetic variability and improve selection (Seichter *et al.*, 2012). Recent advances in gene sequencing now allow determination of the complete genome of an animal (Werner, 2011), but, due to costs and bioinformatic complexity, this technology has not been widely employed.

A look into the future of technology

Sequencing of the first draft of the human genome required three years of intensive effort and cost an estimated 1 billion USD. Currently, high-throughput sequencing of a single genome can be achieved in one week for approximately $18,000 USD. With the advent of nanopore technology, by which single molecules of DNA can be deciphered as they pass through a tiny channel, a new paradigm of sequencing is on the not too distant horizon (Pennisi, 2012b). This technology will revolutionize sequencing. Current micropore methods depend on protein pores, but solid state, i.e. silicon wafer pores, are in development (Pennisi, 2012a). The great advantage of these procedures is that they eliminate the need for DNA replication before sequencing and for the reassembly of the genome after sequencing, both steps that are required by current technology. With scale-up, nanopore technology is expected to be able to reveal an entire genome sequence

in 15 min (Pennisi, 2012a). Costs will fall precipitously with the reduction in reagents, equipment and procedures. We can therefore imagine that the capability for sequencing the genome of sires or dams will soon become available at the bench in every laboratory. As sequencing technology can also be used with complementary DNA, this revolution will extend to transcriptomic investigation at much lower cost than microarray or RNAseq procedures.

It is clear that these technical advances, along with currently available technology, will result in an avalanche of information within the next decade. On the genomic side, it will allow for exploration of complex traits that produce variant milk production, meat quality, feed conversion, adaptation to climate and disease resistance, among others that can then be used for selection. The value of this research can be seen in results using current technology in which genomic regions responsible for variation in the immune response to foot-and-mouth disease in cattle have been partially mapped (Leach *et al.*, 2010). In silico analysis (information available on internet databases) has revealed multiple pathways and regions associated with resistance to internal parasites in domestic animals (Sayre and Harris, 2012). Microarray and RNAseq methods have provided the capability for global transcriptomic analysis, and many studies have been conducted addressing livestock reproductive issues such as the early bovine embryo development (Clemente *et al.*, 2011), embryo-uterine interactions at maternal recognition of bovine pregnancy (Mamo *et al.*, 2011) and epigenetic modifications in multiple tissues (Wu *et al.*, 2012). The clear challenge is, and will continue to be, the bioinformatic analysis of the masses of data that can be so easily collected. We can expect further evolution of bioinformatic tools will be required to exploit the future technology. In addition, a systems biology approach, in which there is integration of experimental, theoretical and computational methods to address dynamic interactions between structure and function, will be essential for understanding of the large genomic and transcriptomic databases that are generated (Quinn and Kohl, 2011).

We can also expect further advances on the stem cell front, as this is an active area of current research. No functional sperm nor ova have yet been produced from pluripotent stem cells, although stem cells have been shown to differentiate into primordial germ cells, and there is some indication of oogonial formation (Lokman and Moore, 2010). Oocyte-like cells have been derived from porcine skin, but have not been shown to mature to competent oocytes (Dyce *et al.*, 2011). It is not unreasonable to predict that these advances will lead to in vitro production of gametes from stem cells. Indeed, it may be possible to produce embryos in vitro from gametes produced completely outside of the gonads. This uncoupling of *in vivo* gametogenesis from embryo production will allow rapid genetic gain to be achieved in livestock, where progress has been impeded by long generation intervals.

Transgenesis or targeted modification of the genome is routine only in a few strains of mice, based on germline transmission of genetically modified embryonic stem cells (Bradley *et al.*, 1992). Following years of intensive research, the embryonic stem cell method has been established for production of transgenic rats (Kawamata and Ochiya, 2011). The first transgenic livestock were produced more than twenty-five years ago by insertion of the transgene into zygotic pronuclei (Kues and Niemann, 2011), but success rates remain discouragingly low. While several other methods for transfer of foreign DNA have been employed, it would appear that somatic cell nuclear transfer is currently the most promising method for livestock transgenesis. Insertion of the transgene into the nucleus to be transferred ensures that the offspring will be genetically modified (Rodriguez-Martinez, 2012). The low success rate of cloning technology, due primarily to the pre-and post-implantation losses (Kohan-Ghadr *et al.*, 2011) renders it impractical for routine use. Nonetheless, we can anticipate improvements in coming years in transgenic animal production that can then be employed toward numerous ends, from insertion of genes to improve production, to provide disease resistance, to alter behavior, or, to produce complex pharmaceuticals in milk.

What are the constraints on research in reproduction in large animals?

It should be abundantly evident that livestock research is essential to address the coming food and environmental crisis. Currently available and emerging technology to explore and exploit reproductive biology has the potential to increase production, reduce dairy herd size without compromising milk yield, reduce environmental contamination and increase nutritional efficiency. For the most part, this message has not been heard by the agencies that fund reproductive investigations. In a recent policy forum editorial, Roberts *et al.* (2009) report what they describe as a dismal state of funding for basic research in livestock. They chronicle correlated trends in decline in the number of scientists in this field and in students and postdoctoral fellows being trained in fundamental research employing large animal models. In Canada, there has been a decline in funded programs for large animal research and recent government moves have reduced the number of scientists in agricultural research centers. This is an alarming trend, given the demonstrated need for improvement in animal production.

Livestock research is, and rightly should be, constrained by considerations for animal welfare. Much of the technology employed, such as semen collection and embryo collection and transfer, is non-invasive in

large animals. Hormone treatments and hormone releasing vaginal devices are relatively innocuous in terms of animal discomfort, but oocyte retrieval and follicle ablation require some interventions with more lasting effects (McEvoy *et al.*, 2006). A major welfare issue is the effects of in vitro embryo manipulation and cloning on gestation in bovids. As noted above, 70% of cloned embryos are lost, those that are born may exhibit developmental anomalies, including large calf syndrome that causes, among other consequences, dystocia. Post-natal survival of offspring is often compromised (McEvoy *et al.*, 2006). The potential benefit of animal biotechnology must be balanced against the problems these methods cause for the animals. This is true not only for research, but also for commercial implementation of technology.

The third constraint to rapid advance is the narrowness of our training and our skill sets. It is clear that graduate student research projects must be goal-oriented and highly focused. Nonetheless, and as indicated above, the technological solutions to the problems will require a panoply of skills, from in depth understanding of reproductive processes through bioinformatics, to the capacity to synthesize and integrate information. Multidisciplinary teams will be required to collect, process, analyze, make sense of and implement the large datasets that are emerging from new technology. Our current training paradigms and research funding programs do not include the global approaches required to meet the needs of this century.

What can Brazil contribute?

Brazil is emerging as a major site for livestock production with an estimated 209.5 M cattle, 38.9 M swine and 32.5 M tons of milk produced each year (http://www.sidra.ibge.gov.br/bda/). It ranks second in the world behind India in exports of beef, 1.5 M tons expected in 2012 alone (http://www.fas.usda.gov). Brazil is among the most biologically diverse countries in the world. Indeed, livestock production in Brazil occurs under a wide variety of climatic and regional conditions, from the tropical climes of Amazonas to the Pampa-like pastures of Rio Grande do Sul and the semi-arid region of the Northeast. To survive and to be economically viable, animals have adapted, not only to heat and humidity, but also with a resistance to ecto- and endoparasites and to infectious and tick-borne diseases. European breeds under these conditions are at best inefficient in growth and milk production, and in some regions, unable to survive. As an example of adaptation, the *Bos indicus* breeds are more resistant to the debilitating tick-borne disease caused by the protozoan parasite, *Theria annulata* than are *Bos taurus* breeds (Glass *et al.*, 2012). The study in question revealed a genomic difference in the response macrophages to inflammation, and differential expression of genes in the bovine major immunohistocompatibility complex (Glass *et al.*, 2012). Likewise, genomic polymorphisms have been identified associated with thermotolerance in Holstein cattle (Li *et al.*, 2011). It can therefore be seen that the genomic information present in Brazilian livestock represents an enormous resource for addressing genomic differences. The rapidly evolving genomic tools described above will permit identification of the genes and gene clusters involved. As transgenic technology matures, it will become possible to transfer genes for thermotolerance or disease resistance to animals that are more productive in temperate climes, but lack the ability to survive in tropical or other conditions. Alternatively, it will become possible to recognize the genes that confer increased milk production, multiple births, feed efficiency or meat quality, and to transfer these to less productive, but heat and disease resistant animals.

Brazil, the world's sixth largest economy, is emerging as a major force in world science. The last 10 yr have seen a doubling in the number of graduate degrees awarded along with a near tripling in the number of scientific papers published, and a tenfold increases since 1980. The biotechnology industry in Brazil is burgeoning supported by both public and venture capital funding (http://www.cebrap.org.br). Animal health and agriculture represent nearly one fourth of the biotechnology activity currently underway in Brazil.

It is therefore expected that Brazil will make major contributions to unraveling the mysteries of reproductive biology in livestock. This is due to the existence of the technology, the intellectual capital, the diversity in genome of the animal populations in Brazil and the progressive and innovative spirit that pervades the country.

References

Avent ND, Madgett TE, Maddocks DG, Soothill PW. 2009. Cell-free fetal DNA in the maternal serum and plasma: current and evolving applications. *Curr Opin Obstet Gynecol*, 21:175-179.

Balmford A, Green R, Phalan B. 2012. What conservationists need to know about farming. *Proc R Soc B Biol Sci*, 279:2714-2724.

Baruselli PS, Ferreira RM, Sales JN, Gimenes LU, Sa Filho MF, Martins CM, Rodrigues CA, Bo GA. 2011. Timed embryo transfer programs for management of donor and recipient cattle. *Theriogenology* ,76:1583-1593.

Bo GA, Cutaia L, Peres LC, Pincinato D, Marana D, Baruselli PS. 2007. Technologies for fixed-time artificial insemination and their influence on reproductive performance of *Bos indicus* cattle. *Soc Reprod Fertil Suppl*, 64:223-236.

Bradley A, Hasty P, Davis A, Ramirez-Solis R. 1992. Modifying the mouse: design and desire. *Biotechnology*, 10:534-539.

Clemente M, Lopez-Vidriero I, O'Gaora P, Mehta JP, Forde N, Gutierrez-Adan A, Lonergan P, Rizos D. 2011. Transcriptome changes at the initiation of elongation in the bovine conceptus. *Biol Reprod*, 85:285-295.

de Graaf SP, Beilby KH, Underwood SL, Evans G, Maxwell WM. 2009. Sperm sexing in sheep and cattle: the exception and the rule. *Theriogenology*, 71:89-97.

de Leeuw N, Hehir-Kwa JY, Simons A, Geurts van Kessel A, Smeets DF, Faas BH, Pfundt R. 2011. SNP array analysis in constitutional and cancer genome diagnostics--copy number variants, genotyping and quality control. *Cytogenet Genome Res*, 135:212-221.

Dyce PW, Shen W, Huynh E, Shao H, Villagomez DA, Kidder GM, King WA, Li J. 2011. Analysis of oocyte-like cells differentiated from porcine fetal skin-derived stem cells. *Stem Cells Dev*, 20:809-819.

Foley JA. 2011. Can we feed the world; sustain the planet? *Sci Am*, 305:60-65.

Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber JS, Johnston M, Mueller ND, O'Connell C, Ray DK, West PC, Balzer C, Bennett EM, Carpenter SR, Hill J, Monfreda C, Polasky S, Rockstrom J, Sheehan J, Siebert S, Tilman D, Zaks DP. 2011. Solutions for a cultivated planet. *Nature*, 478:337-342.

Friel S, Dangour AD, Garnett T, Lock K, Chalabi Z, Roberts I, Butler A, Butler CD, Waage J, McMichael AJ, Haines A. 2009. Public health benefits of strategies to reduce greenhouse-gas emissions: food and agriculture. *Lancet*, 374:2016-2025.

Glass EJ, Crutchley S, Jensen K. 2012. Living with the enemy or uninvited guests: functional genomics approaches to investigating host resistance or tolerance traits to a protozoan parasite, Theileria annulata, in cattle. *Vet Immunol, Immunopathol*. doi.org/10.1016/j.vetimm.2012.03.006. Epub ahead of print.

Godbout S, Verma M, Larouche JP, Potvin L, Chapman AM, Lemay SP, Pelletier F, Brar SK. 2010. Methane production potential (B0) of swine and cattle manures--a Canadian perspective. *Environ Technol*, 31:1371-1379.

Ireland JJ, Smith GW, Scheetz D, Jimenez-Krassel F, Folger JK, Ireland JL, Mossa F, Lonergan P, Evans AC. 2011. Does size matter in females? An overview of the impact of the high variation in the ovarian reserve on ovarian function and fertility, utility of anti-Mullerian hormone as a diagnostic marker for fertility and causes of variation in the ovarian reserve in cattle. *Reprod Fertil Dev*, 23:1-14.

Kawamata M, Ochiya T. 2011. Gene-manipulated embryonic stem cells for rat transgenesis. *Cell Mol Life Sci*, 68:1911-1915.

Kearney J. 2010. Food consumption trends and drivers. *Philos Trans R Soc Lond B Biol Sci*, 365:2793-2807.

Kitzman JO, Snyder MW, Ventura M, Lewis AP, Qiu R, Simmons LE, Gammill HS, Rubens CE, Santillan DA, Murray JC, Tabor HK, Bamshad MJ, Eichler EE, Shendure J. 2012. Noninvasive whole-genome sequencing of a human fetus. *Sci Transl Med*, 4:137ra76.

Kohan-Ghadr HR, Fecteau G, Smith LC, Murphy BD, Lefebvre RC. 2011. Endocrine profiles of somatic nuclear transfer-derived pregnancies in dairy cattle. *Theriogenology*, 76:911-920.

Kues WA, Niemann H. 2011. Advances in farm animal transgenesis. *Prev Vet Med*, 102:146-156.

Lamb GC, Dahlen CR, Larson JE, Marquezini G, Stevenson JS. 2010. Control of the estrous cycle to improve fertility for fixed-time artificial insemination in beef cattle: a review. *J Anim Sci*, 88:E181-E192.

Leach RJ, Craigmile SC, Knott SA, Williams JL, Glass EJ. 2010. Quantitative trait loci for variation in immune response to a Foot-and-Mouth Disease virus peptide. *BMC Genet*, 11:107.

Li Q, Han J, Du F, Ju Z, Huang J, Wang J, Li R, Wang C, Zhong J. 2011. Novel SNPs in HSP70A1A gene and the association of polymorphisms with thermo tolerance traits and tissue specific expression in Chinese Holstein cattle. *Mol Biol Reprod*, 38:2657-2663.

Lokman M, Moore H. 2010. An artificial sperm - next year or never? *Hum Fertil*, 13:272-276.

Mamo S, Mehta JP, McGettigan P, Fair T, Spencer TE, Bazer FW, Lonergan P. 2011. RNA sequencing reveals novel gene clusters in bovine conceptuses associated with maternal recognition of pregnancy and implantation. *Biol Reprod*, 85:1143-1151.

Mapletoft RJ, Bo GA. 2011. The evolution of improved and simplified superovulation protocols in cattle. *Reprod Fertil, Dev*, 24:278-283.

McEvoy TG, Alink FM, Moreira VC, Watt RG, Powell KA. 2006. Embryo technologies and animal health - consequences for the animal following ovum pick-up, in vitro embryo production and somatic cell nuclear transfer. *Theriogenology*, 65:926-942.

McMichael AJ, Powles JW, Butler CD, Uauy R. 2007. Food, livestock production, energy, climate change, and health. *Lancet*, 370:1253-1263.

Pennisi E. 20012a. Genome sequencing. Going solid-state. Science, 336:536

Pennisi E. 2012b. Genome sequencing. Search for pore-fection. *Science*, 336:534-537.

Place SE, Mitloehner FM. 2010.: Contemporary environmental issues: a review of the dairy industry's role in climate change and air quality and the potential of mitigation through improved production efficiency. *J Dairy Sci*, 93:3407-3416.

Quinn TA, Kohl P. 2011. Systems biology of the heart: hype or hope? *Ann N Y Acad Sci*, 1245:40-43.

Roberts RM, Smith GW, Bazer FW, Cibelli J, Seidel GE, Jr, Bauman DE, Reynolds LP, Ireland JJ. 2009. Research priorities. Farm animal research in crisis. *Science*, 324:468-469.

Rodriguez-Martinez H. 2012. Assisted reproductive techniques for cattle breeding in developing countries: a critical appraisal of their value and limitations. *Reprod Domest Anim*, 47(suppl 1):21-26.

Sayre BL, Harris GC. 2012. Systems genetics approach reveals candidate genes for parasite resistance from quantitative trait loci studies in agricultural species. *Anim Genet*, 43:190-198.

Seichter D, Russ I, Rothammer S, Eder J, Forster M, Medugorac I. 2012. SNP-based association mapping of the polled gene in divergent cattle breeds. *Anim Genet*, doi: 10.1111/j.1365-2052.2011.02302.x. Epub ahead of print.

Sills ES, Alper MM, Walsh AP. 2009. Ovarian reserve screening in infertility: practical applications and theoretical directions for research. *Eur J Obstet Gynecol Reprod Biol*, 146:30-36.

Werner T. 2011. Next generation sequencing allows deeper analysis and understanding of genomes and transcriptomes including aspects to fertility. *Reprod Fertil Dev*, 23:75-80.

Wu S, Li RW, Li W, Li CJ. 2012. Transcriptome characterization by RNA-seq unravels the mechanisms of butyrate-induced epigenomic regulation in bovine cells. *PLoS One*, 7:e36940.