Fuller W. *Bazer*
Animal Science
Texas A&M University

Reproductive Biology of Domestic Animals: Linkages with Veterinary and Human Medicine

This paper will discuss opportunities for linkages between research in animal agriculture and research directly related to human medicine. Results of research in animal agriculture have affected certain aspects of clinical medicine for many years. The application of biotechnology to human and veterinary medicine involves many techniques used in reproductive biology and applied in animal agriculture. Among these are embryo transfer, gene injection, use of embryonic stem cells for introduction of genes and cloning of embryos. Gene transfer in animals is being used to evaluate the value of transgenic animals in animal agriculture. Transgenic animals are also being used for the study of specific genetic defects. Production of transgenic animals has received much attention recently. This technique represents only one approach which can be used not only to examine questions of interest to the biomedical community, but will also help establish linkages between animal agriculture, veterinary medicine and human medicine. Naturally occurring diseases in livestock must be examined for their value as models for human disease. Regulatory proteins normally secreted by the conceptus (embryo/fetus and associated membranes) may have unique therapeutic value for certain diseases. In addition, regulatory proteins secreted in significant amounts may be associated with normal development of the conceptus, but with various disease states in adults. Although it may be useful to create transgenic animals as models for specific diseases, we must also focus on naturally occurring diseases affecting animals that are common to humans.

Reproductive biology, in particular the study of pregnancy in livestock, provides numerous opportunities to address questions of biology with application to both veterinary and human medicine. Pregnancy and associated development of the conceptus seems analogous to compressing events of a lifetime into a period of gestation. Because of extremely rapid development of the conceptus, basic questions relative to proliferation and differentiation of cells, cell-cell interactions and regulation of gene expression can be

addressed. Results impact our knowledge of wound healing, cancer biology, tissue transplantation biology, immunology, developmental^ regulated expression of genes, endocrine regulation of maternal and fetal-placental expression of genes, the hematopietic system and so forth. It is not surprising that investigators from numerous disciplines have petitioned for the opportunity to study fetal-placental tissues of humans. Again, understanding normal events and mechanisms associated with the reproductive process will directly impact animal agriculture and the biomedical community.

GENE TRANSFER IN HUMAN AND NONHUMAN ANIMALS

It is clear that techniques such as embryo transfer, gene injection, cloning of embryos and related technologies have always been done first in animals so that proven methods can later be applied to human and veterinary medicine. Gene transfer in animals is now being evaluated critically in a number of species and with a number of genes. The problems laboratories face in attempting to produce transgenic animals include poor expression, lack of expression or over expression of genes of interest, and in some cases, lack of incorporation of injected genes. Documentation of these problems in animals and development of technologies to overcome them must be through animal experimentation before these techniques become useful to clinical medicine. In some cases, tissue specific expression is essential. This is also being studied extensively in animals in which expression of genes are being restricted to the mammary gland and the gene products are being harvested from milk. These technologies will eventually impact animal agriculture and medicine, but experimentation with animals will continue to be central to this research.

ALTERNATIVES TO TRANSGENIC ANIMALS

An alternative to development and use of transgenic animals is the identification of animal models which have naturally occurring diseases and/or metabolic disorders that provide natural linkages between animal agriculture and the biomedical community. Although rodents or other small laboratory animals are commonly used, domestic animals should be used as models whenever possible so that scientific breakthroughs will impact animal health, human health and production animal agriculture. For example, sheep are used extensively as the animal model for studies of basic questions pertaining to pregnancy, perinatology and neonatology in humans in departments of pediatrics and departments of obstetrics and gynecology. Results from such research directly impacts the base of knowledge available to clinicians to improve survival and well-being of neonatal humans and animals. Importantly, the same information benefits production animal agriculture.

Trophoblast In terferons

Acquired immunodeficiency syndrome (AIDS) is having devastating effects on human health throughout the world. Similar lentivirus-induced diseases

affect cattle, sheep, horses, goats, primates and cats which makes them excellent animal models for studies of the etiology, prevention and treatment of human AIDS. A potential therapeutic drug for AIDS is recombinant leukocyte interferon alpha. The trophoblast of conceptuses of some species (e.g., ruminants and humans) produce unique interferons which may be especially useful for treatment of AIDS and AIDS–like diseases because the trophoblast interferons appear to lack the undesirable toxic side effects of leukocyte interferons.

Type I trophoblast interferons (tIFN) of sheep, goats and cows are biochemical signals for maternal recognition of pregnancy which may be useful for enhancing fertility in animal agriculture; however, they may also have therapeutic value in human and veterinary medicine (Bazer and Johnson, 1991). The tIFNs have high amino acid sequence homology with both interferon alpha I ($IFN_{\alpha I}$) and interferon alpha II ($IFN_{\alpha II}$) or omega interferon (IFN_{Ω}) which are produced by white blood cells. The tIFNs produced by sheep, cow and goat conceptuses are very similar to each other in structure and biological activity. A gene for human tIFN (htIFN) that has 85 to 87 percent homology with sheep tIFN (otIFN) has been cloned from a human placental cDNA library (Whaley et al., 1991).

The tIFNs have potential therapeutic value because they are interferons that inhibit cellular proliferation (Pontzer et al., 1991), exert antiviral effects (Pontzer et al., 1991) and regulate the immune system (Newton et al., 1989). Evaluation of the potential therapeutic value of tIFNs requires sufficient amounts of pure protein for clinical studies. A synthetic gene for sheep tIFN is being used in yeast and bacterial expression systems to produce recombinant sheep tIFN (rotIFN) that is identical to natural otIFN in terms of its amino acid sequence and biological activities (Ott et al., 1991). The antiviral activity of this tIFN is as potent as that of known recombinant leukocyte interferons from humans (rhIFN) and cattle (rbIFN), but sheep recombinant tIFN does not exert cytotoxic effects characteristic of treatment with $rhIFN_{\alpha}$ and $rbIFN_{\alpha}$ (Pontzer et al., 1991; Bazer et al., 1989). Exposure of human and feline peripheral lymphocytes infected with human immunodeficiency virus (HIV) and feline immunodeficiency virus (FIV) respectively, to sheep tIFN inhibited replication of the viruses, but did not exert cytotoxic effects on the infected cells when used at concentrations up to 200,000 antiviral units per ml. However, $rhIFN_{\alpha}$ and $rbIFN_{\alpha}$ exerted significant cytotoxic effects at only 1,000 to 5,000 antiviral units per ml.

The tIFNs also have antiproliferative effects on cells that is equivalent to or greater than that of $rbIFN_{\alpha}$ and $rhIFN_{\alpha}$ (Pontzer et al., 1991) and may be useful in the treatment of cancers. When anticellular activities of sheep tIFN, $rbIFN_{\alpha}$ and $rhIFN_{\alpha}$ were compared using human amnion (WISH) and Madin–Darby bovine kidney (MDBK) cells, all inhibited proliferation of the cells. However, sheep tIFN was more effective at lower dosages and, at high

dosages (50,000 antiviral units per ml), otIFN more effectively blocked cell proliferation without adverse effects on cell viability. At the same concentrations of antiviral activity, $rbIFN_a$ caused substantial cell death (Pontzer et al., 1991; Bazer et al., 1989).

Human patients having steroid-dependent mammary tumors respond to treatment with ^-interferons because of increased receptors for progesterone and decreased receptors for estrogen in tumor cells (DeCicco et al., 1988). In the pregnant uterus of sheep, tIFN stabilizes receptors for progesterone while decreasing receptors for estrogen (Ott et al., 1992). The health of many women is affected adversely by estrogen-dependent tumors of the mammary glands and reproductive tract. Because estrogen-dependent tumor growth depends on the presence of cellular receptors for estrogen, tIFNs have potential therapeutic value because they inhibit synthesis of cellular receptors for estrogen which should prevent estrogen-dependent growth of the tumors.

Humans suffering from infection with HIV or diseases such as hairy cell leukemia are willing to consider lifelong therapy with rhIFN; however, chronic treatment with rhIFN results in development of resistance to the effects of currently available recombinant rhIFN (Tamm et al., 1987). In addition, high doses of rhIFN produce intolerable fever and chills, anorexia, weight loss and fatigue (Oldham, 1985) and may also cause seizures (Janssen et al., 1990). Interferons have both immuno-enhancing and cytotoxic effects; therefore, therapeutic doses are chosen which favor the immuno-enhancing effects. In contrast, tIFNs act through receptors on the uterine epithelium which are in direct contact with the conceptus and are exposed to as much as 40 million units of antiviral activity per 24 hours without cytotoxic effects. These tIFNs have unique "cell friendly" properties which may make them especially desirable therapeutic agents for use in animal agriculture, veterinary medicine and human medicine.

A number of diseases affecting livestock result from infections by lentiviruses of the family *Retroviridae.* These include, ovine progressive pneumonia virus (OPPV), caprine arthritis-encephalitis virus (CAEV), bovine immunodeficiency-like virus (BIV), equine infectious anemia (EIA), feline immunodeficiency virus (FIV) and simian immunodeficiency virus (SIV) (Haase, 1986). Diseases caused by OPPV, CAEV and BIV, for example, are uniquely suited for testing the therapeutic value of tIFNs in the control of lentivirus-induced diseases because conceptuses of each of these species secrete tIFN. These animal models must be studied to assess the therapeutic value of tIFNs in preventing or ameliorating vertical transmission of lentiviruses (i.e., via the placenta), and horizontal transmission (i.e., animal to animal), as well as the efficacy of tIFNs in treating infected fetuses and adult sheep, goats and cattle.

Hematopoietic System

Another protein isolated from reproductive tissues of domestic farm animals which has potential application to veterinary and human medicine is uteroferrin. Uteroferrin is a purple-colored, progesterone-induced glycoprotein secreted by uterine endometrial epithelium of pigs (Bazer et al., 1991). Uteroferrin can also be purified from human term-placenta. During pregnancy, uteroferrin is transported from uterine secretions into the fetal-placental circulation and is targeted to reticuloendothelial cells of the fetal liver, the major site of hematopoiesis in fetal pigs. Uteroferrin, from pig uterus is a tartrate-resistant acid phosphatase with many properties in common with the Type 5 tartrate-resistant acid phosphatase in human placenta, chondrocytes of osteoclastic bone tumors, spleens of patients with hairy cell leukemia, as well as Gaucher's and Hodgkin's diseases (Ketcham et al., 1985). In addition, uteroferrin has characteristics similar to those for purple acid phosphatases from normal bovine, rat, mouse and pig spleen, as well as bovine milk, bovine uterine secretions, equine uterine secretions and rat bone. Uteroferrin from pig uterus and human placenta is a hematopoietic growth factor having granulocyte-erythrocyte-monocyte/macrophage-megakaryocyte colony forming unit (CFU-GEMM) activity that affects differentiation of primitive nonadherent hematopoietic stem cells from pig and human bone marrow (Bazer et al., 1991).

Uteroferrin from pig uterine endometrium and human placenta (Ketcham, 1988), appears to influence hematopoiesis during fetal life. In adult humans, however, the presence of the Type 5 tartrate-resistant acid phosphatase, which has high amino acid sequence homology with uteroferrin, is indicative of abnormal function of cells associated with hematopoietic tissues (Ketcham et al., 1985) in such diseases as hairy cell leukemia. It is not clear why there is such abundant secretion of uteroferrin by pig uterus and human term-placenta during the course of a normal pregnancy while the apparently identical protein is associated with pathological conditions in adults, e.g., hairy cell leukemia. An understanding of this paradoxical situation requires further studies to determine the precise role of uteroferrin in hematopoiesis. Animal agriculture, veterinary medicine and human medicine will benefit from collaborative efforts to understand the role of this protein in health and disease.

Reproductive Management

Research with farm animals has contributed significantly to the development and use of clinical methods to enhance fertility through the use of techniques for *in vitro* fertilization, embryo transfer, embryo culture, endocrine therapy, cloning of embryos and cryopreservation of sperm, ova and embryos. These techniques are used to enhance fertility in human and nonhuman animals. Methods for contraception or reducing fertility are also based, in large part,

on results of experiments with domestic livestock. Functional sterility is desired by many humans to limit family size and in animals it may be used to control growth of populations in general or in a specific geographical area. Research continues to develop methods which render one functionally sterile, but are reversible. Progress in development of immunological methods for achieving fertility control continue and will impact programs designed to offer safe and effective alternatives for regulation of fertility in human and nonhuman animals.

DEVELOPMENT OF ANIMAL GENETIC MODELS OF DISEASE

Development of animal models to study human disease often raises ethical and/or animal welfare issues. However, these issues must be considered in light of the tremendous positive impact disease research has had in the past and will undoubtedly provide for the future for both human and nonhuman animals. Diseases of humans and animals must be understood if the adverse impact of those diseases are to be ameliorated or eliminated. The possibility of experimentation with naturally occurring or transgenic animal models expressing diseases, or metabolic disorders, is essential to the biomedical community. Otherwise, we could not adequately address issues of health, disease and reproductive management that our increasingly global society will present during the next century.

REFERENCES

Bazer, F.W., H.M. Johnson and J.K. Yamamoto. 1989. Effect of ovine trophoblast protein-1 on replication of the feline and human immunodeficiency viruses. *Biol. Reprod.* 40: Suppl 1: 63.

Bazer, F.W. and H.M. Johnson. 1991. Type I conceptus interferons: Maternal recognition of pregnancy signals and potential therapeutic agents. *Am. J. Reprod. Immunol.* 26:19-22.

Bazer, F.W., D. Worthington-White, M.F.V. Fliss and S. Gross. 1991. Uteroferrin: A progesterone-induced hematopoietic growth factor of uterine origin. *Exp. Hematol.* 19:910-915.

DeCicco, F., G. Sica, M.T. Benedetto, G. Ciabattoni, F. Rossiello F., A. Nicosia, G. Lupi, F. Iacopino, S. Mancuso and S. Dell'Acqua. 1988. 'In vitro' effects of (3-interferon on steroid receptors and prostaglandin output in human endometrial adenocarcinoma. /. *Steroid Biochem.* 30:359-362.

Haase, A.T. 1986. Pathogenesis of lentivirus infections. *Nature.* 322:130-136.

Janssen, J.L.S., L. Berk, M. Vermeulen and S.W. Schalm. 1990. Seizures associated with low dose a-interferon. *The Lancet.* 336:1580.

Ketcham, C.M., G.A.Baumbach, F.W. Bazer and R. M. Roberts. 1985. Immunological, chemical and enzymatic similarities between uteroferrin and Type 5 acid phosphatase isozyme from human leukemia hairy cells. /. *Biol. Chem.* 260:5768.

Ketcham, C.M. 1988. The human Type 5, tartrate resistant acid posphatase: purification, characterization and molecular cloning. Doctoral Dissertation. University of Florida, Gainesville.

Newton, G.R., J.L. Vallet, P.J. Hansen and F.W. Bazer. 1989. Inhibition of lymphocyte proliferation by ovine trophoblast protein-1 and a high molecular weight glycoprotein produced by preimplantation sheep conceptus. *Am. /. Reprod. Immunol. Microbiol.* 19:99-107.

Oldham, R.K. 1985. Biologicals for cancer treatment: interferons. *Hospital Practice.* 20:71-91.

Ott, T.L., G. Van Heeke, M.H. Johnson and F.W. Bazer. 1991. Cloning and expression in S. cerevisiae of a synthetic gene for the Type I trophoblast interferon ovine trophoblast protein-1: purification and antiviral activity. /. *Interferon Res.* 11:357-364.

Ott, T.L., Y. Zhou, M.A. Mirando, C. Stevens, J.R Harney, T.F. Ogle and F.W. Bazer. 1992. Changes in progesterone and estrogen messenger ribonucleic acid and protein during maternal recognition of pregnancy in ewes. *J. Mol. Endocrinol,* (submitted).

Pontzer, C.H., F.W. Bazer and H.M. Johnson. 1991. Antiproliferative activity of a pregnancy recognition hormone, ovine trophoblast protein-1. *Cancer Res.* 51:5304-5307.

Tamm, I., B.R. Jasny and L.M. Pfeffer. 1987. Antiproliferative action of interferons. In *Mechanisms of Interferon Actions, Vol II.* L.M. Pfeffer, ed., pp 26-57. CRC Press, Inc., Boca Raton, FL.

Whaley, A.E., R.S. Carroll, K.P. Nephew and K. Imakawa. 1991. Molecular cloning of unique interferons from human placenta. *Biol. Reprod.* 44 (Suppl.l):186.