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FINAL REPORT

PROJECT NO. US-2643-95

Role of Placental Lactogen in Sheep

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Project Title: Role of Placental Lactogen in Sheep

Investigators

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See Achievements

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Final Scientific Report
Role of Placental Lactogen in Sheep
Fuller W. Bazer, Arie Gertler and Elisha Gootwine

ABSTRACT

Central problems in sheep and dairy cattle production are reproductive failure due to embryonic/fetal mortality and low birth weights, especially in prolific breeds, and reduced milk yields which adversely affect neonatal survival and economy of production. The sheep placenta expresses lactogenic (ovine placental lactogen, oPL) and somatogenic (ovine placental growth hormone, oGH) hormones. Our research has focused on the biological roles of oPL and oGH in function of the uterine endometrium during gestation and the mammary gland during pregnancy and lactation. Major conclusions were that: (1) immunization of prepubertal ewes against oPL resulted in increased birth weights of their lambs and their milk production during lactation; (2) neither oPL nor oGH had an antiluteolytic effect on uterine endometrium to affect lifespan of the corpus luteum; (3) only sequential exposure of the progesterone stimulated uterus to oIFN τ and oPL or oGH increased endometrial gland proliferation and secretory protein gene expression; (4) oPL signals through a homodimer of ovine prolactin receptor (PRL-R) and heterodimer of oPRL-R and growth hormone receptor (GH-R); (5) exogenous recombinant oPL and oGH stimulated mammary gland development and milk yield during lactation; and (6) mutation of oPL and oGH was used to define specific biological effects and a rational basis for design of a specific receptor agonists or antagonists. This project was very productive in elucidating basic biological effects of oPL and oGH on intracellular signal transduction pathways, uterine development and secretory function, as well as mammary gland development and lactogenesis. We determined that immunization of prepubertal ewes against roPL increased birth weights of their lambs, especially those born as twins and triplets, as well as enhanced lactational performance. These studies significantly extended our knowledge of uterine and fetal-placental physiology and provided a foundation for new strategies to enhance reproductive and lactation efficiency. Based on these results, the major achievements were: 1) creation of a practical and cost effective management tool for producers to increase reproductive performance, neonatal survival, and milk yield of ewes in commercial flocks; and 2) define, for the first time, biological effects of oPL on endometrial functions and gene expression by uterine gland epithelium.

ACHIEVEMENTS

Significance of main scientific achievements or innovations

The BARD project US-2643-95 was very productive in elucidating novel and basic biological effects of oPL and oGH on intracellular signal transduction pathways, uterine development and secretory function, and mammary gland growth, differentiation and function.

Objective 1. Is oPL the pregnancy maintenance signal in sheep which reinforces or extends effects of interferon tau (oIFN τ), the pregnancy recognition signal? Results from three experiments indicated that neither oPL nor oGH affected the endometrial luteolytic mechanism [Spencer et al. 1999]. Two experiments determined effects of progesterone, estrogen, roPL and roGH on expression of endometrial gland (GE) secretory proteins, including uterine milk proteins (UTMP) and osteopontin (OPN). Both oGH and oPL increased expression of UTMP in uterine GE, but only oPL stimulated expression of OPN. oPL stimulated proliferation of deep GE, whereas oGH only stimulated proliferation of middle GE. Effects of oPL and oGH on endometrial function required pre-exposure of the endometrium to progesterone and IFN τ . This is the first evidence that oPL and oGH have paracrine effects on uterine endometrium to increase GE proliferation and gene expression for secreted proteins that may enhance fetal-placental growth and development in ewes.

Objective 2. Analyze expression of oPL and oGH genes during pregnancy and study the role of oPL by *in vivo* immunoneutralization of its activity. Gene mapping indicated that oPL evolved from the PRL gene and that two copies of oGH gene are expressed by sheep placenta [Ofir and Gootwine 1997; Gootwine and Yossefi 1998]. Daily injections of oGH and oPL to growing lambs and lactating ewes [Liebovich et al. 2000] revealed similar somatogenic activities to increase ($P<0.05$) growth of lambs (10-25%) and increase milk production of ewes by 55% (oGH) and 25% (oPL). Two experiments, one with prolific non-dairy Booroola-Assaf sheep and one with Assaf dairy sheep in which ewe lambs were immunized against roPL [Leibovich et al. 2000] indicated that increased ($P<0.01$) birth weights of lambs born to immunized ewes by 0.2, 0.5 and 0.9 kg per lamb for singles, twins and triplets, respectively. Immunized ewes also produced more milk (22%, $P<0.05$) than control ewes during the first 110 days of lactation. Higher oPL bioactivity and, since up to 50% of the oPL was antibody-bound, longer half-life of bioactive oPL, and increased production of oPL by placentae, may account for effects of the immunization protocol. Thus, immunization of pre-pubertal ewes against oPL can be used to maximize production efficiency of ewes in commercial flocks.

Objective 3. Investigate the structure-function relationships of oPL to determine domains responsible for diverse activities mediated through three different receptor types and identify oPL analogues with activities targeted to specific biological functions. Three oPL analogues, characterized for *in vitro* activity, revealed that mutations at positions 185 and 133 abolished or selectively decreased heterologous somatogenic activity, whereas lactogenic activity was affected less. One oPL mutant (G133R) is an antagonist to somatogenic

activity of oPL [Helman et al. 1998]. Interestingly, ovine, bovine and caprine PLs were not active in homologous somatogenic assays using cells transfected with the GH receptor (oGH-R), although oGH and oPL bound with similar affinities to the oGH-R extracellular domain. Thus, ruminant PLs antagonize oGH activity in homologous systems, because they can not homodimerize oGH-Rs, and, in heterologous systems, they act as agonists [Herman et al. 1999]. Gel-filtration and surface plasmon resonance kinetic analysis documented that oPL heterodimerizes PRL-R and GH-R. These results are the first to describe a unique mechanism of oPL (and other ruminant PLs) action, as no specific oPL receptor has been cloned or purified. Thus, *in vivo* activity of oPL on cells expressing both GH-R and PRL-R may be transduced primarily via receptor heterodimers. A study of mammatrophic effects of oPL and oGH in pseudopregnant ewes revealed that roGH and roPL increased insulin-like growth factor one (IGF-I) in blood and milk yield [Kann et al. 1999], but only roPL increased lobuloalveolar development and β -casein production.

Agricultural and/or economic impacts of the research findings. Results of these studies clearly indicate that oPL and oGH affect uterine, fetal-placental and mammary gland biology that directly enhances conceptus development and lactation in ewes. Immunization of prepubertal ewes against roPL markedly increased birth weights of lambs, especially those born as twins and triplets, and enhanced milk production. These novel findings define a practical cost-effective management protocol for immediate use by sheep producers to increase production efficiency and profitability. In the next 5 years, we expect to implement management practices that improve reproductive and lactational performance of ewes through cost-effective use of recombinant lactogenic and somatogenic hormones. In the next 10 years, we expect to transfer appropriate technologies to the marketplace, farms and ranches, for use of exogenous oPL and oGH to improve birth weights and survival rates of offspring born to prolific ewes, nannies and cows, and to increase milk yield of all ruminants. The outcome is expected to increase milk and milk product yields to markedly improve production efficiency in animal agriculture. These goals will have a significant positive economic impact on producers throughout the world as small ruminants increasingly become a major source of animal protein in developing countries.

Details of cooperation. During the grant period, the multidisciplinary research team had a synergistic, complementary and supportive relationship that enabled the investigators to complete all proposed objectives. Each member of the research team contributed specific expertise to allow for a multitude of experiments to be conducted. The scientists are committed to joint research endeavors, and this commitment is strengthened by travel. Indeed, Dr. Gootwine visited Dr. Bazer's laboratory at Texas A&M University in 1997. A student from Dr. Gertler's laboratory, Mr. Asael Herman, worked in the laboratories of Drs. Bazer and Spencer at Texas A&M University for 3 months in the Fall of 1998 and 2000. In 1999, Drs. Bazer and Spencer visited the laboratories of Drs. Gertler and Gootwine in Israel to discuss and present BARD-funded experimental results and to plan future endeavors. The

cooperation between each investigator was complementary, necessary and provided critical knowledge for development of management tools for use by producers to enhance production efficiency of small ruminants.

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