Final BARD Scientific Report

Overall summary and statistics

BARD Project Number: US-3199-01CR

Date of Submission of the report: December 1, 2005

Project Title: Placental Lactogen Enhances Production Efficiency in Sheep

Investigators

Institutions

Principal Investigator (PI):

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Co-Principal Investigator (Co-PI):

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Collaborating Investigators:

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Keywords *not* appearing in the title and in order of importance;

Lamb; birth weight; milk production;

Abbreviations commonly used in the report, in alphabetical order:

Budget: IS: \$ 150,000

US: \$ 150,000

Total: \$ 300,000

Signature
Principal Investigator

Signature
Authorizing Official, Principal Institution

Publication Summary (numbers)

	Joint IS/US authorship	US Authors only	Israeli Authors only	Total
Refereed (published, in press, accepted) BARD support acknowledged	2	3	2	7
Submitted, in review, in preparation	0	0	2	2
Invited review papers	0	3	2	5
Book chapters	0	1	0	1
Books	0	0	0	0
Master theses	0	0	0	0
Ph.D. theses	0	1	0	1
Abstracts	4	2	2	4
Not refereed (proceedings, reports, etc.)	0	0	0	0

Postdoctoral Training: None.

Cooperation Summary (numbers)

	From US to Israel	From Israel to US	Together, elsewhere	Total
Short Visits & Meetings	1	1	2	4
Longer Visits (Sabbaticals)	0	1	0	2

Description Cooperation:

Each member of the research team contributed specific expertise to allow for a multitude of experiments to be conducted. Dr. Gootwine conducted the animal experiments outlined in Objectives 1 and 2, while Dr. Spencer conducted the animal experiments outlined in Objective 3. Drs. Gootwine and Spencer routinely communicated data and planning for experiments. Dr. Gertler prepared gram quantities of recombinant hormones from bacteria and checked them for biological activity and purity. The hormones were sent to Drs. Spencer and Gootwine for animal studies or stored for future use.

Patent Summary (numbers)

	Israeli inventor only	US inventor only	Joint IS/US inventors	Total
Submitted	0	0	0	0
Issued (allowed)	0	0	0	0
Licensed	0	0	0	0

Abstract

The key objectives of this BARD project were to: (1) study long-term effects of immunization of prepubertal ewes against recombinant ovine placental lactogen (roPL) on subsequent birth weights of their lambs and their milk production; (2) optimize the anti-roPL immunization protocol using adjuvant preparations acceptable to producers and regulatory agencies; and (3) determine the physiological mechanism(s) whereby immunization against oPL increases fetal growth and development and mammogenesis. These objectives were based on key findings from a previous BARD project that: (a) immunization of ewes against roPL increased lamb birth weight and ewe milk production during lactation; (b) roPL and recombinant ovine growth hormone (roGH) increased the proliferation and differentiated function of endometrial glands that, in turn, would enhance uterine secretions necessary for fetal and placental growth; and (c) exogenous roPL and roGH stimulated mammogenesis and milk production during lactation. The BARD projects address central problems in sheep production, including reproductive failure due to embryonic/fetal mortality, low birth weight of lambs especially in prolific breeds, and reduced milk yields which affect neonatal survival. The sheep placenta secretes both lactogenic (oPL) and somatogenic (oGH) hormones. The receptors for those hormones are present in the fetus and placenta as well as maternal uterus, and mammary gland. Our research has focused on determining the biological role of these placental hormones in development and differentiation of the uterus during gestation and the mammary gland during pregnancy and lactation. Studies conducted in the current BARD project indicated that the effects of anti-roPL immunization were variable in ewes and that commercially available and widely acceptable adjuvant preparations were not effective to produce high anti-roPL titers in pre-pubertal ewes. In the non-prolific Rambouillet ewe in Texas and in the Awassi and the Assaf in Israel, anti-roPL immunization increased lamb birth weight; however, the magnitude of this effect and the inherent variability precluded our ability to determine the physiological mechanism of how the immunization increases fetal growth. Collectively, our findings suggest that anti-roPL immunization is not currently feasible as an easy and efficacious tool for the producer to increase flock reproductive and production efficiency. The variability in response of individual ewes to anti-roPL immunization likely includes modifying the recombinant hormone and the type of adjuvant used for the immunization. In particular, the oPL may need to be modified to ensure maximum antigenicity in a broad range of breed types. Nonetheless, the investigators continue to collaborate on identifying fundamental mechanisms that can be improved by genetics or management to enhance the efficiency of uteroplacental function and, in turn, fetal growth and development. High prolificacy is a desirable trait in intensive sheep production systems. One of the main limitations of using prolific breeds of sheep is that increased litter size is associated with low birth weights and increased mortality of lambs. Further, low birth weight is associated with an increased propensity for adult diseases and decreased production efficiency. Indeed, our recent studies find that the birth weights of lambs born in large litters can be improved by both genetics and management. Future cooperative research will continue to focus on reproductive efficiency of sheep that have broader implications for improving production efficiency in all types of ruminant livestock.

Achievements

Significance of main scientific achievements or innovations.

Objective 1: Study long-term effects of immunization of prepubertal ewes against ovine placental lactogen (oPL) on subsequent birth weights of their lambs and their milk production. Our previous study reported that average birth weights of lambs born to ewes immunized against roPL were higher ($P < 0.01$) than for lambs born to control ewes by 10%, 17% and 39% for those born as singles, twins and triplets, respectively. Immunized ewes produced 19% and 33% more milk ($P < 0.02$) than the control ewes in the first 3.5 months of the first and second lactations, respectively (Liebovich et al., *Anim Reprod Sci* 2000; 64:33-47). Our working hypothesis was that following immunization at pre-pubertal age, production of endogenous oPL in each successive pregnancy will serve as natural booster for the immunization status.

During the present grant period, 10 immunization trails were conducted in Israel and Texas where birth weight of lambs and ewes' milk production was investigated in immunized ewes (n=418) and their contemporary control ewes (n=461). Only in two trials, lambs born to immunized ewes were significantly heavier at birth than lambs born to control ewes.

Milk production was followed in Awassi ewes up to the third lactation in two trails. In both trails milk production of the immunized ewes was higher, especially in the third lactation. However, the effect was not statistically significant.

			Milk production (LSM$\pm$SE)		
			1st	2nd	3rd
Treatment	Year	n	lactation	lactation	lactation
Control	2000	44	378 $\pm$ 18	573 $\pm$ 25	577 $\pm$ 31
oPL immunized (FCA)	200	9	425 $\pm$ 58	657 $\pm$ 75	637 $\pm$ 73
Control	2002	29	353 $\pm$ 26	528 $\pm$ 22	478 $\pm$ 38
oPL immunized (ISA)	2002	34	363 $\pm$ 24	554 $\pm$ 35	554 $\pm$ 38

Objective 2: Optimize the oPL immunization protocol using adjuvant preparations acceptable to producers and regulatory agencies. In our original publication (Liebovich et al., *Anim Reprod Sci* 2000; 64:33-47), an immune response against roPL was achieved using Freund's Complete Adjuvant (FCA). This potent adjuvant can cause undesired formation of granulomas and abscesses at immunization sites, thereby rendering it unacceptable for use in commercial flocks where immunized ewes will ultimately be used for human consumption. In the present project, ewe lambs from five flocks in 2000 and four flocks in 2002 were immunized against roPL as described previously, using Montanide ISA 206 (Seppic, Paris, France) adjuvant, or FCA. Control ewes were not immunized. The Montanide ISA 206 adjuvant is advertised as providing a rapid and durable immune response and is approved for commercial use in sheep. A mixture 1:1 monomer and oligomer forms of roPL was used for the immunization in most locations. Blood samples were collected from control and immunized ewes 3 weeks after the second booster and anti-roPL titers were determined.

Generally, the ISA adjuvant proved to be less potent than FCA. In the 1998 trial, a difference of about 10-fold was observed in anti-roPL titers between the two locations where the experiments took place. In Years 2000 and 2002, ewes immunized against roPL developed immunity; however, larger variability in titers between the immunized groups was observed. The variability between the groups in the anti-roPL titers is not related to breed (Awassi or Assaf) or location. The unexplained differences may result from differences in batches of the immunogen or be attributed to the immunization procedure. The present results confirm that an immune response against roPL can be induced by immunizing ewe lambs against roPL. The observed titers for the FCA adjuvant were similar to those reported before. However, the Montanide ISA 206 adjuvant, which is commercially used for sheep vaccinations, was found to be less potent than FCA in inducing anti-roPL immune activity. The basis for the variability between ewes in developing anti-roPL immune activity has to be further investigated. Effect of roPL immunization on lamb birth weight and ewe milk production was observed in some, but not in all flocks. This may be partly explained by the variability in immunity of sheep at the different locations.

Objective 3. Determine the physiological mechanism(s) whereby immunization of prepubertal ewes against oPL increases lamb birth weights and ewe milk production. In Years 2001 and 2002, prepubertal Rambouillet ewe lambs (n=100) at Texas A&M University were immunized against roPL (n=50) using FCA or adjuvant only (n=50) using established procedures. Lambs born as a singleton (n=67) were heavier ($P<0.05$) in anti-oPL immunized as compared to control ewes (oPL versus control: 4.5 ± 0.1 versus 2.2 ± 0.1 kg). Lambs born as a twin (n=10) tended to be heavier ($P<0.10$) in anti-oPL immunized as compared to control ewes (oPL versus control: 4.0 ± 0.4 versus 3.2 ± 0.7 kg). In the winter of 2004, all control and anti-oPL immunized ewes were bred after a mild superovulation. The mild superovulation resulted in a mean litter size of 2.7. No effect of anti-oPL immunization was detected ($P>0.10$) on lamb birth weight. The same population of immunized ewes was to be utilized for the remainder of Objective 3 to determine the physiological mechanism of how anti-roPL immunization increases fetal growth and ewe milk production. However, the magnitude of the effect combined with the variability in the immunization effect would preclude the investigators from easily identifying the underlying physiological mechanism whereby anti-oPL immunization affects lamb birth weight. Indeed, our recent studies suggest that genetic and environmental factors affect lamb birth weight and may likely interfere with the anti-oPL effect in some ewes. In particular, we found that the *FecB* mutation which is segregating in the Beit Dagan flock affects lamb's birth weight.

Although the anti-roPL immunization remains problematic, great potential exists in using recombinant hormone treatment in neonatal ewes to permanently modify uterine development, thereby enhancing adult reproductive efficiency (Spencer and Bazer, *J Anim Sci* 2004; 82 (E. Suppl.):E4–E13). Indeed, treatment of ewe lambs with recombinant ovine prolactin (roPRL) from birth to 8 weeks of age increased the development of the endometrial glands of the uterus (Carpenter et al., *Endocrinology* 2003; 144:110-120). In high prolificacy ewes, the ability of roPRL treatment to increase uterine development and, thus, uterine capacity might be an effective tool to increase reproductive and production efficiency. The collaborating investigators remain committed to understanding the basic mechanisms of uterine development that should allow them to develop rational genetic or epigenetic tools to increase uterine capacity and production efficiency.

Details of cooperation: The multidisciplinary research team had a synergistic, complementary and supportive relationship that enabled the investigators to complete most of the 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. Gertler visited Dr. Spencer's laboratory at Texas A&M University in 2004 and 2005. Dr. Gertler supplied all of the recombinant hormones used by Drs. Gootwine and Spencer. At present, Dr. Gootwine is on sabbatical at Texas A&M University and working with Drs. Tom Spencer and Fuller W. Bazer to elucidate the differences in oGH variants produced by the ovine placenta as well as the fundamental mechanisms of placental efficiency. Further, the investigators continue to correspond by e-mail and meet at scientific meetings to discuss research objectives and findings.

List of Publications:

Carpenter KD, Gray CA, Noel S, Bazer FW, Gertler A, Spencer TE. Prolactin regulation of neonatal ovine uterine gland morphogenesis. *Endocrinology* 2003; 144:110-120.

Gertler A, Djiane J. Mechanism of ruminant placental lactogen action: molecular and in vivo studies. *Mol Genet Metab* 2002; 75:189-201.

Gootwine E. Placental hormones and fetal-placental development. *Animal Reproduction* 2004; 82-83:551-566.

Gootwine E. High variability in the rate of decline in birth weight as litter size increases in sheep. *Animal Science* 2005; 81:393-398.

Gootwine E, Rozov A, Bor A, Richer S. Carrying the FecB (Booroola) mutation is associated with lower birth weight and slower post weaning growth rate for lambs and lighter mature body weight for ewes. *Reprod Fertil Dev* 2005; (submitted).

Noel S, Herman A, Johnson GA, Gray CA, Stewart MD, Bazer FW, Gertler A, Spencer TE. Ovine placental lactogen specifically binds endometrial glands of the ovine uterus. *Biol Reprod* 2003; 68:772-780.

Spencer TE, Bazer FW. Uterine and Placental Factors Regulating Conceptus Growth in Domestic Animals. *J Anim Sci* 2004; 82 (E. Suppl.):E4-E13.

Spencer TE, Johnson GA, Burghardt RC, Bazer FW. Progesterone and placental hormone actions on the uterus: insights from domestic animals. *Biol Reprod* 2004; 71:2-10.

Appendix (technical information supporting the research findings):

- (1) Published papers.
- (2) Submitted papers.