

PROPOSAL TITLE PAGE

Partnering with Higher Education for International Development
2001 REQUEST FOR PROPOSALS

1. Coordinating Institution in the U.S. : **University of Wisconsin - Madison**

2. U.S. Project Director Name : **J.J. Rutledge**

Title : **Professor**

Address : **1675 Observatory Drive**

Department of Animal Sciences

City : **Madison**

State : **WI**

Zip : **53706**

Telephone : **(608) 263-6993**

Fax : **(608) 262-5157**

E-mail : **rutledge@calshp.cals.wisc.edu**

3. Partnering Higher Education Institution in USAID-assisted country:

Universidad Nacional del Altiplano Puno, Peru

4. Institutional Partner(s) Project Director(s) Name (add sheets as necessary):

(Please refer to attached sheet)

Title _____

Address _____

City _____ Country _____ Postal Code _____

Telephone _____

Fax _____

E-mail Address _____

5. Name of Proposed Project: **Evaluation of Yak Crossbreds as High Altitude
Milk Cows on the Altiplano**

6. USAID Goal and Strategic Objective(s) or Special Initiative Addressed : **To encourage broad-based economic growth and agricultural development, to build human capacity through education and training**

7. Funds Requested of ALO (\$100,000 maximum for duration of project) \$ **99,958**

8. Matching Funds and Resources from Institutional Partners \$ **81,559**

9. Project Partners other than higher education institutions (e.g., community organizations, state or local government, business, industry) **CIRNMA, BOMED, Inc., Estación Experimental ILLPA-Puno**

10. Executive Summary of the partnership activity and the anticipated results (250 words; attach separate sheet)

11. This ALO grant program intends to make use of a private Internet listserve as a means by which important information can be transmitted to project directors. All U.S. Institutional partners project directors must subscribe.

Do the project directors have direct Internet access? X Yes ___No

12. Please list any other U.S. federal agencies where you have submitted this particular proposal or any similar/overlapping application for funding. Also, if this proposal is a component of a larger project that has been funded or for which funds are being sought, please explain. **None**

Executive Summary of the partnership activity and the anticipated results

The Altiplano of Peru is a high, arid grassland with limited opportunity for plant agriculture. Poverty is abundant. Camelids thrive but are not very

productive; productive bovids are ill adapted to high altitudes. In similar ecosystems in Central Asia herders for thousands of years have created a high altitude adapted milking cow by crossing yak with cows. About one-half of each herd must be purebred (yak and cow) to sustain the system as reproductive excess in bovids is low. Three universities (U.S. Chile and Peru), a Peruvian NGO, a US Corporation, Peruvian Agricultural Ministry and an International agency formed a consortium to research the feasibility of using modern embryo biology to emulate the indigenous knowledge solution of Asian herders. Oocytes taken from Wisconsin spent dairy cows will be fertilized with yak sperm and the resulting embryos will be air-transported to Peru for transfer to cows. Resulting heifers will be evaluated as milk cows in the Altiplano ecosystem. Note no purebreds are maintained, only productive hybrids. Hybrids will be born initially at experiment stations, but in the second year transfers will be made to privately owned cows. Plant agriculture and pasturage by cattle ceases at higher elevations, which are now used only for camelid production; we hope to demonstrate that the hybrid can be a productive milk cow in these areas. This should create new employment opportunities as for example in cheese making. University and certificate students from Peru and other Andean countries will be fully involved with the husbandry and testing of the hybrids.

Narrative:

Description of the Proposed Partnership: The University of Wisconsin – Madison, Madison, WI, Universidad Nacional del Altiplano, Puno, Peru, Universidad de la Frontera, Temuco, Chile, Instituto Nacional de Investigación Agraria (INIA) of the Peruvian Ministry of Agriculture, Centro de Investigación de Recursos Naturales y Medio Ambiente (CIRNMA), Puno, Peru and BOMED, Inc., Madison, WI have discovered mutual interests in research and education centering on the problem of lack of a high-altitude adapted milk cow with focus on the Altiplano. We propose to pool resources to investigate the practicality of

using modern biotechnological tools to enable an indigenous knowledge solution to this problem. Wisconsin and Chilean universities contribute technology, BOMED, Inc. will loan some equipment, Peruvian University and Ministry will provide animals and husbandry and CIRNMA (an NGO) will contribute expertise in outreach and participatory research in the Altiplano.

USAID Goals and Strategic Objectives: We address the USAID goals 1) to encourage broad-based economic growth and agricultural development and 2) to build human capacity through education and training, and USAID-Peru strategic objective: Increased Incomes of the Poor.

Rationale

A primary motivation for this proposal is widespread poverty found in the Altiplano region of South America. Although we propose to work in the Department of Puno, Peru, our work should be directly applicable to the Altiplano regions found elsewhere in Peru as well as those in Bolivia, Chile and Argentina. According to data supplied by our partner CIRNMA, the annual per capita income in the Department of Puno is \$460, about 42 % of the national; the infant mortality rate is 90 per thousand; almost one-quarter are illiterate (one-third of women); 80 % of the houses have no electricity or no water service or are lacking both; and nearly 60 % of the inhabitants were assessed as undergoing chronic malnutrition. There is the problem of migration from the Department to other areas and migration within the Department from rural areas to urban centers. These indicators reveal the extent of the problem and fully justify the Peruvian's declaration of a war against poverty in this region. We believe that research and education will be vital in combating poverty. A basic premise of this research is that there exists much similarity between the South American Altiplano agroecosystem and that of the Tibetan and Qinghai plateaus of Central Asia, and that solutions to problems in one ecosystem might be exploited in the other. The problem is lack of a milk cow well adapted to high altitudes; the solution comes from indigenous knowledge of Himalayan herders; and the economically sustainable implementation comes from recent developments in embryo biology.

Comparison of Altiplano and Central Asian Agroecosystems: The two agroecosystems lie at similar latitudes relative to the equator: in Asia from about 27 to 35° N and in South America from 15 to 30° S. They have similar altitudes, somewhat above 3,800 m. Both can be characterized as shrub, sparse grass and wastelands¹. Soils in Asia are Mountain soils of Tundra zone with lithosols while those in South America are Mountain soils of the Latosolic soil zones with lithosols². Both are arid having average annual rainfall amounts less than 25 cm. In both areas there evolved large herbivores especially adapted to these conditions. In South America the guanaco (*Lama guanicoë*), a camelid, and in Asia the yak (*Bos grunniens*) and the zebu (*B. indicus*), both bovids, were domesticated by native peoples.

A basic premise of this research is that there exists much similarity between the South American Altiplano agroecosystem and that of the Tibetan and Qinghai plateaus of Central Asia, and that solutions to problems in one ecosystem might be exploited in the other. The problem is lack of a milk cow well adapted to high altitudes; the solution comes from indigenous knowledge of Himalayan herders; and the economically sustainable implementation comes from recent developments in embryo biology.

Production by Large Herbivores in the Altiplano: Today in the Altiplano extensive use is made of the llama and alpaca (domesticated guanacos) for meat and fiber production, but not milk, and of criollo cattle for meat, milk, and manure production as well as traction and as a conservator of wealth. Near Lake Titicaca extensive use is made of Brown Swiss crossbreds; these are less well adapted to the ecosystem than the criollo but are more productive. Criollo cattle result from introductions by the Spanish about 500 years ago, although much crossing with modern European breeds has been done and few animals having only inheritance from the original imports can be found. All the bovines are *Bos taurus*. In any event none of the large herbivores currently in South America are both adapted and productive in the harsh Altiplano environment. Llama and alpaca are not very productive although well adapted; criollo are

¹ Reader's Digest Atlas of the World

² Rand McNally Atlas

somewhat adapted, especially at lower elevations and more productive than both domesticates of guanaco, at least they have a wider suite of productive functions. Criollo crosses are poorly adapted but somewhat productive. High grades and purebred European breeds tend to be limited to irrigated areas at lower altitudes where the full effects of the Altiplano ecosystem are ameliorated.

Purebred *Bos taurus* animals moved to high altitudes often experience so-called brisket disease which is a congestive heart failure. This disease is similar to the human disease: high altitude pulmonary edema. Brisket disease is sometimes experienced in high mountain pastures of the Western United States and is characterized by edema in brisket, jugular vein distention and high neonatal calf mortality. Twenty percent of the Brown Swiss and Angus calves die shortly after birth at the Centro Experimental Estación at Chuquibambilla from this disease; criollo calves do not die. Generally *B. taurus* is not well adapted to high altitude, although some can survive after acclimatization.

Production by Large Herbivores in Central Asian Plateaus: Today in the high arid pasturelands of Central Asia extensive use of yak x zebu F_1 crosses is made since the F_1 combines some of the productive ability of cattle with some of the adaptive attributes of yak (Rong-Chang, 1985). The two parental species evidence complementarity, and the F_1 exhibits considerable heterosis. Making this cross is an ancient practice first reported to the western world by Marco Polo (Epstein, 1974). In 1999 Professor Rutledge visited with farmers near Zhongdian, Yunnan Province, PRC, where alpine cattle rearing furnished the main income. Animals were individually owned but communally herded. One quarter of the cow herd was the common Chinese Yellow cow, one quarter were yak cows and the remainder of the females were F_1 crosses. Both yak and Chinese Yellow bulls were used to produce straightbreds and F_1 's although hybrids by the yak are preferred. F_1 heifers were said to be twice as valuable as either yak or Yellow cow heifers. Because of brisket disease Chinese Yellow cattle are not pastured as high as yak or F_1 crosses. Yak and F_1 crosses are safely pastured up to 5000 m.

Since there is little reproductive excess in cattle populations half of the cattle resources of the village visited had to be allotted to unadapted (Chinese Yellow) or unproductive (yak) species. F₁ males were used for meat [they are invariably sterile, Haldane (1922)] as were progeny of the F₁ cows. No backcrosses were kept for milk production as there is apparently much recombination load in crosses beyond the F₁ of these two species as was found for taurus and zebu crosses (Rutledge, 2000). It is a testament to the utility of the F₁ that so many resources are devoted to its production. The secondary research thrust of this proposal is to evaluate application of technology to avoid maintaining large populations of breeders in the production of F₁ crosses in cattle. In fact, it is technologically feasible to have a production system where the entire population consists of only F₁ females.

Brief Literature Review:

A. Performance of yak x cow hybrids: Our thesis is that yak crosses with temperate breed cattle are known to be adapted to high deserts and should be evaluated for production purposes in the Altiplano. We are not alone in this belief. An Ad-hoc panel authored a National Research Council report (NRC, 1983) entitled Little-known Asian Animals With a Promising Economic Future. Of yak-cattle hybrids they wrote:

“Where pastures occur over areas of greatly varying altitude, the use of hybrid livestock is likely to be more efficient, in biological terms, than the use of yaks or cattle... The hybrid’s milk is intermediate in composition between that of its parents. However, hybrids yield up to 7 kg of milk per day against the yak’s 3 kg. ... Also the female hybrids produce larger quantities of milk than Nepalese zebu cows on the same pastures. They also produce a calf each year, while under normal herd management yaks tend to produce a calf every 2 years. ... Research to determine the most productive hybrids by crossing different yak and cattle “breeds” in areas with different environmental conditions could be extremely useful.”

A portion of this proposal is to do essentially what the Ad Hoc Panel suggested. Our partnership will evaluate Holstein x Yak crosses for their

suitability as milk cows on the Altiplano. Our methods of producing the hybrids for evaluation will be heavily technology dependent in an effort to obtain a sustainable, low-cost production methodology avoiding the expense of keeping unproductive or unadapted purebreds. The Israeli scientist (Epstein, 1974) who spent much of his career characterizing genetic resources and diversity in the tribe bovine wrote:

"Female hybrids of yak and cattle are fertile when mated with a bull of either parent stock, but male hybrids are sterile ... The hybrids excel in hardiness, size, strength, working ability and milk production ... Their live weight is approximately 18 percent higher than midparental values ... Hybrid cows reach sexual maturity earlier and yield more milk than yak cows. ... The milk yields of the parent breed of cattle have a marked influence on the yields of their hybrid daughters."

Rong-Chang (1985) reviewed yak rearing in China. Some 12 million head (85 percent of the world's total) are reared on the Qinghai and Tibetan Plateaus. He wrote:

"China has a long history of crossbreeding yaks and ordinary cattle. ... The F₁ hybrids of the different species are not only adaptable to the environmental conditions of high mountain grasslands but their production performance and feed efficiency are improved."

R.W. Phillips traveled extensively in the Tibetan highlands during World War II as part of the USDA contribution to the war effort. He concluded (Phillips, 1948): "It is possible that they could serve a useful purpose in other parts of the world where similar environmental conditions prevail." Clearly, in the environment where adapted, the hybrid is the bovine equivalent of the mule.

B. In vitro bovine embryo production: In the last two decades or so the in vitro production of cattle embryos has progressed from impossibility to a mature technology (Gordon, 1994). Scores of laboratories exist that have this capability, and the in vitro production of cattle embryos has even entered high-school curricula. In vitro embryo production is an integral part of some production systems in Europe, North America and Japan (Rutledge, 1998).

Briefly, cattle cumulus oocyte complexes can be aspirated from graafian follicles on the surface of ovaries. A convenient source of ovaries is an abattoir where this organ is part of the offal. Oocytes are matured or ripened in vitro in a culture medium; this takes about 24 hours. Sperm thawed from liquid nitrogen temperatures are centrifuged through a Percoll gradient to remove diluent and dead sperm. Sperm are added to the oocytes at a concentration found optimal for the individual bull being used (usually $\sim 1 \times 10^6/\text{ml}$), and the gametes are coincubated for 24 hours. The presumptive embryos are then moved to culture medium where they reside for 7 days. At the end of this period about 25 percent of the ova fertilized are at the blastocyst stage of development. At this stage they can be non-surgically transferred to the uterus of a recipient where about half will, nine months later, be born. In vitro bovine embryo production has been a routine procedure of the Rutledge laboratory since the middle 1980's. The Director of Research at our partner, BOMED, Inc., Madison, WI was one of the pioneers of bovine embryo production.

Wisconsin has a major meatpacking industry based on slaughter of spent dairy cows and fed Holstein steers. Rutledge (1997) calculated the genetic value for milk production of an oocyte from a spent dairy cow. It averaged approximately that of cows still on the farms in their third lactation. Thus they are of high genetic merit, and this is the resource we intend to exploit. McHugh and Rutledge (1998) showed that frozen-thawed yak sperm could be used to fertilize abattoir-derived eggs with a reasonable rate of embryo production. Ambrose et al. (2000) demonstrated the feasibility of shipping pre-implantation bovine embryos fresh using a portable incubator and air transport. Thus all of the needed technologies are in place and have been demonstrated to be workable.

Literature Cited:

Ambrose, J.D., M. Drost, R.L. Monson, J.J. Rutledge, M.L. Leibfried-Rutledge, M.J. Thatcher, T. Kassa, M. Binelli, P.J. Hansen, P.J. Chenoweth and W.W. Thatcher. 1999. Efficacy of timed embryo transfer with fresh and

- frozen in vitro produced embryos to increase pregnancy rates in heat-stressed dairy cattle. *J. Dairy Sci.* 82: 2369-2376.
- Epstein, H. 1974. Yak and chauri. *World Anim. ReD.* Bishop, K.J. Eilertson and E.G. Crichton. 2001. Bovine viral diarrhea virus (BVD) and anti-BVD antibodies in samples of pooled bovine follicular fluid. *Theriogenology* 55: 376 (abstr).
- Gordon, I. 1994. *Laboratory Production of Cattle Embryos.* CAB Int'l, Wallingford, UK
- Haldane, J.B.S. 1922. Sex ratio and unisexual sterility in hybrid animals. *J. Genet.* 12, 101-109.
- McHugh, J.A. and J.J. Rutledge. 1998. Heterologous fertilization to characterize spermatozoa of the genus *Bos*. *Theriogenology* 50: 185-193.
- NRC. 1983. Little-known Asian Animals with a Promising Economic Future. Ad Hoc Panel of the Advisory Committee on Technology Innovation, National Research Council. U.S.A., National Academy Press, Washington, D.C.
- Phillips, R.W. 1948. *Breeding Livestock Adapted to Unfavorable Environments.* FAO Agricultural Studies No. 1. Washington, D.C.
- Rong-Chang, Z. 1985. China: the yak. *World Anim. Rev.* 54: 24-29.
- Rutledge, J.J. 1997. Cattle breeding systems enabled by in vitro embryo production. *International Embryo Soc. Newsletter* 15 (1): 14-18.
- Rutledge, J.J. 1998. Applications of in vitro methodology in tropical dairying. *Proc. 17th Tech Conf. on AI and Reprod. Nat'l Assn Anim Brds.* Sept 25-26 Madison, WI
- Rutledge, J.J. 2000. Greek Temples, tropical kine and recombination load. *Livestk. Prod Sci.* 68: 171-179.

Plan of Work: F₁ cattle -yak hybrids will be made at the University of Wisconsin using oocytes from BOMED, Inc., Madison, WI. They will be transported in the fresh state to Peru for implantation; Dr. Del Campo will coordinate transfers. At the Universidad de Altiplano Experiment Station at Chuquibambilla criollo cows will be used as recipients (40 head of cows have been reserved for this project). Near Puno, embryos will be transferred to 20 Brown Swiss cows at the INIA's

Estación Experimental ILLPA. The resulting heifers will be evaluated for their suitability as milk cows in the arid, high altitude and nutrient poor pasture environments of the Altiplano. Further, since ILLPA has control over some very high pastures (up to 5000 m) typically suited only for camelids, some of the males will be evaluated for meat production and adaptation at very high altitudes. This should give an indication of the suitability of their sisters for milk production at these high altitudes. The usual sort of animal production data will be collected on all animals.

Direct farmer and student involvement with this project will be encouraged. For example the Universidad de Altiplano Experiment Station has students in attendance year round. These are not only University students, but also students attending a very popular certificate course that requires 8 months in residence. This practicum course attracts students from Bolivia as well as Peru. Our NGO partner, CIRNMA, has much experience in participatory agricultural research working directly with Altiplano farmers. In the second year CIRNMA will coordinate privately owned cows being used for transfer. This will probably occur at two villages that CIRNMA has previously worked with. We anticipate making transfers to our partnership herds as well. Thus we will have hard scientific data from the first and second year transfers and data of a more practical sort from the transfers to privately owned cows.

Outreach and institution building: Part of the outreach effort is allied with the research effort of the paragraph just above. We expect that data collected in evaluation of these hybrids will be part of a Master of Science thesis at the Universidad de Altiplano de Puno. In vitro embryo production technology is crucial to the sustainability of this program. In the first year we propose to bring two or three younger Professors from the Universidad de Altiplano de Puno to the University of Wisconsin for a 3 month intensive course in in vitro embryo production. Also we will adapt a laboratory at the University de Altiplano de Puno for in vitro embryo production. It is our intent to have this laboratory produce at least some of the embryos for the year 2 transfers, and be able to operate independently of Wisconsin, except for advice and routine trouble shooting. Further, since it is not in Peru's best interest to be tied to a single oocyte source we will initiate the technology for importation of oocytes

from New Zealand and/or places in South America (Southern Brazil, Uruguay) where high quality spent dairy cows are available.

Benefits to partners and their missions:

Research and education are central to the missions of the universities involved. For the Universidad de Altiplano, this research will enable training of their faculty in cutting edge technologies in biology of reproduction and provide a focus for continuing collaboration with UW-Madison, particularly the Babcock Institute for International Dairy Research and Development. Once a stable and surplus milk supply is assured for the Altiplano then matters of value-added products, e.g. cheeses, etc., and potential for enhanced employment opportunities will need to be addressed. For UW-Madison the benefits are an increased research arena and exposure of staff and students to global agriculture and industry. For the Chilean University, this project represents a pilot project for applications in the Altiplano of Chile. The Ministry of Agriculture will benefit in access to cutting edge technology and having their research staff work with university personnel. BOMED, Inc. will benefit as this represents a pilot study for applications of reproductive biotechnology to the solution of a class of genetic problems arising with cattle production systems where *Bos taurus* is ill-adapted. CIRNMA will benefit as this project will move its activities into dairying – they have mostly been concerned with agronomic work previously.

Evaluation Plans: There are at least four measures required. First is Can these technologies be applied? Success will be determined by birth of calves of the desired genotypes. Second is Can these technologies be applied in Peru? Success will be determined by birth of calves made by in vitro production methods in Peru. Third is Can these hybrids survive and produce economic quantities of milk in higher areas where such production is impossible or impractical with *Bos taurus*? Success will be determined by challenging the animals to Altiplano conditions and determining milk production. Finally fourth is Will these hybrids be accepted and used by the people of the Altiplano? Frankly, this question will not be answered by this project as insufficient time is available, but good preliminary information will come from the students and staff who will work with these animals.

Key personnel:

Dr. J.J. Rutledge, Department of Animal Sciences, University of Wisconsin, Madison

Dr. Juan Astorga Neyra, Rector, Universidad Nacional del Altiplano, Puno, Peru

Dr. Marcelo Del Campo, Centro de Biotecnología Reproductiva, Universidad de la Frontera, Temuco, Chile

Dr. Roberto Valdivia Fernández, Centro de Investigación de Recursos Naturales y Medio Ambiente, Puno, Peru

Dr. Guido Perez, Universidad Nacional de Altiplano, Puno, Peru

Dr. Rolando Alencastre Delgado, Estación Experimental, Chuquibambilla, Peru

Dr. Saturnino M. Vilca, Estación Experimental ILLPA, Puno, Peru

Dr. Lorraine Leibfried-Rutledge, BOMED, Inc. Madison, WI

Cost-sharing commitments

Generally, UW-Madison will be responsible for making embryos and providing overall coordination on this project. BOMED, Inc. will provide portable incubators for transport of embryos and/or oocytes and council on shipping media, methods of transport, etc. Universidad de la Frontera will provide for preparation of recipient cows, assist in transfer of embryos and matters of cow-side reproductive biology. Universidad de Altiplano and Estación Experimental ILLPA will provide recipient cows and husbandry. They will take major role in data collection. CIRNMA will coordinate outreach efforts and coordinate participatory research with smallholders.

Dissemination Plan: Scientific parts of this work will meet requirements for publication in a scientific journal. Summaries will be included in annual report of The Babcock Institute, annual report from Rutledge to USDA (CRIS), and annual report of Rutledge to W-171 Regional Research Project: Germ Cell and Embryo Development and Manipulation for the Improvement of Livestock. Comparable reports in Spanish will be made in academic circles in South America. Finally, CIRNMA along with Universidad de Altiplano make efforts to release and interpret these results directly to smallholders residing in the Altiplano. We will build a web site to reach other audiences.