NATIONAL ANIMAL GERMPLASM PROGRAM

2005 ANNUAL REPORT

NATIONAL ANIMAL GERMPLASM PROGRAM COLLECTION UPDATE

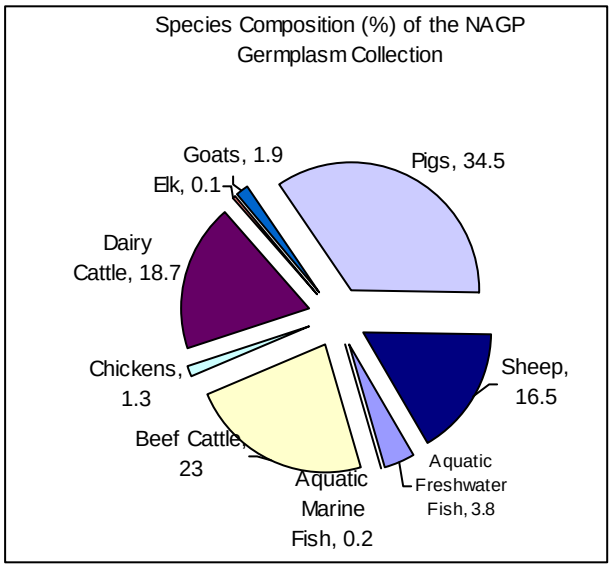
Problem: Animal genetic resources are the underpinning for profitable livestock production. The genetic resources and genetic diversity can be protected by developing cryopreserved reserves for all economically important livestock species. Without such reserves the livestock industry is at risk from a narrowing genetic base, threat of contagious diseases, and bioterrorism. To address the issue of animal genetic resources, conservation requires three simultaneous actions: building cryopreserves, developing an information system to enhance user understanding of the national collection and breed population dynamics, and understanding the genetic diversity present in livestock populations.

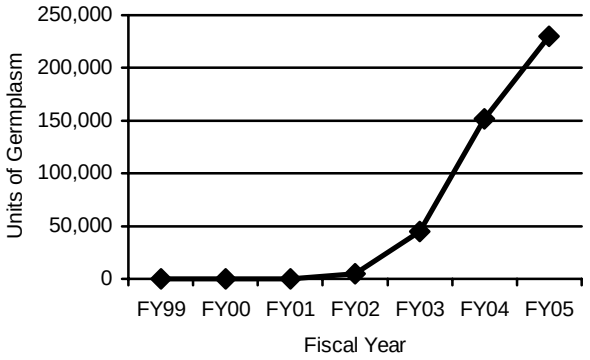
Findings: At the end of fiscal year 2005, the collection contained:

- 99 breeds of cattle, swine, sheep, and goats,
- 82 chicken lines,
- 16 aquatic species,
- samples from more than 5,475 different animals, and
- 243,440 samples of semen, blood, and embryos.

In addition, sufficient germplasm has been collected to meet minimum breed security targets for the following breeds or lines:

- Cattle - Holstein, Jersey, Angus, Hereford, Salers, and Parthenais
- Swine – Yorkshire, Meishan and Danbred
- Chickens - 17 lines of research populations





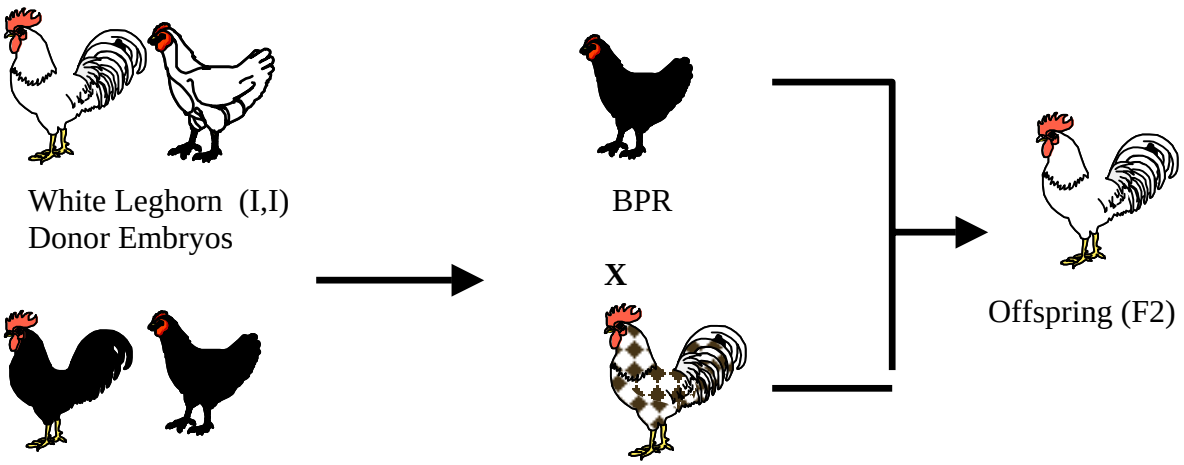
Interpretations and Recommendations: Development of cryopreserved germplasm reserves provides a security backup for the U.S. livestock industry and the American consumer. Although the collection has grown significantly during the past year, significant additions at the breed and within breed levels are still needed.

- H. Blackburn
- P. Purdy

THE USE OF PRIMORDIAL GERM CELLS TO COLLECT POULTRY GENETIC MATERIAL

Problem: The only method for preserving chicken genetic resources is semen cryopreservation. However, there are disadvantages to using cryopreserved poultry semen. These include: storing lines with inbred genetic backgrounds, poor semen quality of inbred lines, only the male genome is saved, and the common cryoprotectant used in the semen freezing process, glycerol, has been proven to be a contraceptive in poultry.

Findings: The use of chicken primordial germ cells (PGCs) as a source for storing poultry genetic material may provide an alternative for cryopreserving poultry genetic resources. PGCs develop outside the embryo, then migrate to the gonad, and develop into mature gametes allowing for access to the poultry genome early in development. Once PGCs have been captured from the donor embryo, they can be injected into a recipient embryo to form a germline chimera. The germline chimera will produce gametes from the injected PGCs.



Interpretations and Recommendations: PGCs can be used to capture both the female and male genome of poultry allowing for the reconstitution of a pure line within two generations. Frozen PGCs can also be used in combination with cryopreserved semen to produce a greater number of pure line offspring. Since PGCs are collected from embryos, eggs can be shipped to one location for collection and storage, eliminating the need for on-site collection or maintenance of flock at the collection location.

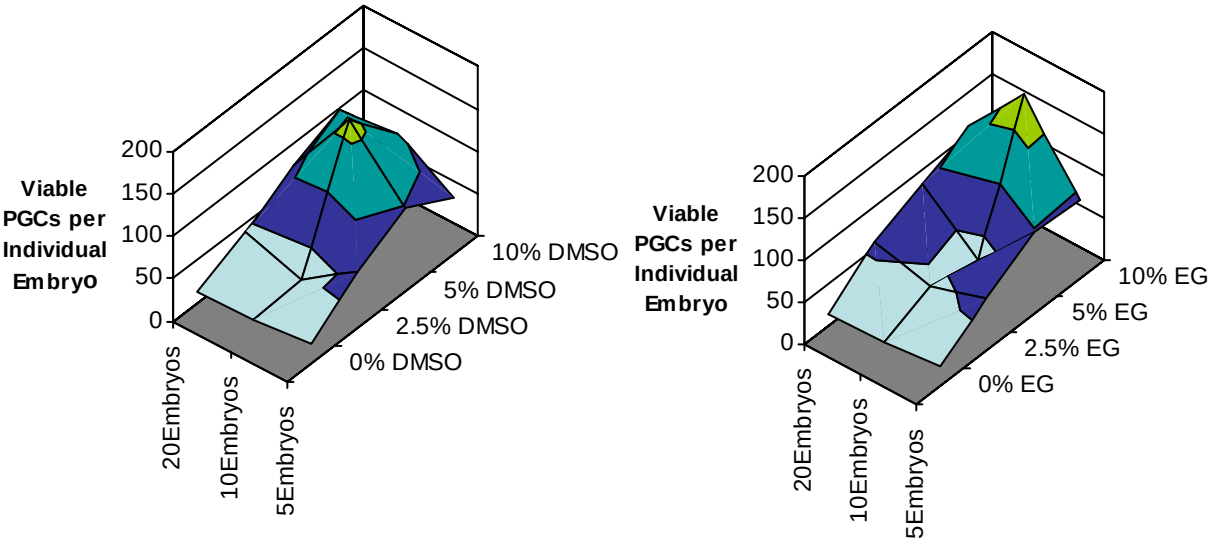
- D. Moore
- H. Blackburn

CRYOPRESERVATION OF CHICKEN PRIMORDIAL GERM CELLS

Problem: Methods for cryopreserving poultry PGCs have not been studied. In order to utilize the ability of PGCs to form germline chimeras for line regeneration, an acceptable method of PGC storage must be developed. Also, the low number of PGCs normally produced per embryo results in a low concentration of cells for freezing.

Findings: Optimal cryopreservation of PGCs has not been studied; therefore, two cryoprotectants [Dimethyl Sulfoxide (DMSO) and Ethylene Glycol (EG)] were used at various concentrations and in combination with three different numbers of embryos donating gonads per cryopreservation straw of PGCs. PGCs were frozen within a gonadal cell suspension to compensate for the low numbers of PGCs normally produced. PGCs were most successfully cryopreserved in 10% Ethylene Glycol with a concentration of 10 embryos donating cells/straw.

The number of viable PGCs when cryopreserved in ethylene glycol (EG) or Dimethyl Sulfoxide (DMSO) with 5, 10, or 20 embryos per straw.



Interpretations and Recommendations: The development of a method for cryopreserving PGCs using the best combination of cryoprotectant and embryos donating cells/straw will allow for the collection of more genetic material from chickens and storing both male and female genomes for line reconstitution.

- D. Moore
- P. Purdy
- H. Blackburn

CONSERVATION AND CHARACTERIZATION OF KAZAK SHEEP BREEDS

Problem: The collapse of the Soviet Union caused a major restructuring of the livestock industry in Kazakhstan. This restructuring is placing the country's animal genetic resources at risk. Prior to this project, Kazakhstan did not have any livestock conservation activities.

Findings: Fifty sheep from five Kazak breeds were sampled (250 head) and moved to a research farm, near Almaty, to establish ex-situ in-vivo breeding flocks (Figure 1). In addition, animal phenotypes were measured and cryopreservation of embryos has been initiated. Ram birth, weaning and yearling weights are presented in Figure 2.

Figure 1. Original locations of Arkharo Merino (1), Sary-Arkinsskaya (2), Edil'baevskaya (3), Chyisskaya (4), and Degeresskaya (5).

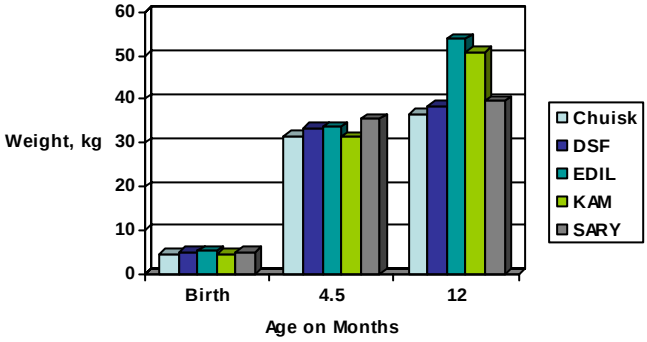


Figure 2. Ram body weights at birth, weaning, and 12 months of age.

Interpretations and Recommendations: Of particular importance is the national recognition this project has received. The Deputy Minister of Agriculture has visited the laboratory and recognized the importance of preserving Kazak animal genetic resources and has decided that the embryos collected by this project are the start of their national conservation program. Furthermore, he formed a committee to initiate animal genetic resource activities and this project's director is the chair of that committee. In addition a FAO officer visited the project and wants to use this project as a case study for a Central Asian workshop on animal genetic resources.

Shown above is Kazakhstan's National Breed of Sheep Edil'baevskaya.

- H. Blackburn
- Y. Toishibekov
- M. Toishibekov

A COMPARISON OF METHODS FOR REMOVING GLYCEROL FROM FROZEN-THAWED ROOSTER SPERM

Problem: The cryoprotectant used for freezing rooster sperm, glycerol, must be removed from the sperm prior to insemination because it is contraceptive. Two potential glycerol removal approaches exist: using Accudenz centrifugation and/or a step-wise dilution method.

Findings: Semen samples from 25 roosters representing 6 lines were thawed and the glycerol was removed by either Accudenz centrifugation or step-wise dilution and centrifugation. After glycerol, removal differences were observed in some motility characteristics (Table 1) and a 66.4 x 10^6 sperm concentration difference between the two methodologies. This is more than half of an insemination dose which is a significant amount of sperm lost using the Accudenz method.

Table 1. Post-thaw characteristics of rooster sperm after step-wise dilution and centrifugation, or centrifugation through Accudenz (12%) for glycerol removal.

Sperm attribute	Accudenz[1]	Step-wise Dilution[1]	Significance
Concentration (sperm/mL)	217.3 ± 24.3 x 10^{6a}	283.7 ± 24.3 x 10^{6b}	.01
Total motility	31% ± 3.1[a]	28% ± 3.1[b]	.02
Progressive motility	12% ± 3.0	10% ± 3.6	.06
Plasma membrane integrity	46% ± 7.4	48% ± 9.3	.17
Track speed (VCL)	77.2 ± 2.5[a]	75.1 ± 2.5[b]	.002

[ab] Indicates a significant difference within a row.

Interpretations and Recommendations: Differences in the motility attributes while significant are biologically minor implying the two processing methods are equal in terms of stress inflicted on the sperm during processing. A loss of sperm during centrifugation is common but the amount of sperm lost with the Accudenz method is greater and therefore reduces the number of inseminations which can be performed per straw of semen. While both approaches may be considered acceptable, the dilution method with its reduced cell loss should be the preferred approach.

- P. Purdy
- H. Blackburn

ASSESSMENT OF THE VIABILITY OF FROZEN-THAWED ROOSTER SPERM

Problem: Preparing rooster sperm for cryopreservation is a rapid process but standard protocols disregard the sperm concentration and therefore the number of insemination doses per semen straw is not known. In addition, the quality of our freezing protocol for rooster sperm has not been previously evaluated. Therefore, our goal was to evaluate post-thaw quality and determine the number of insemination doses on a per straw basis.

Findings: Rooster semen samples (n = 45) from 25 males, representing 6 lines, were frozen and thawed and the sperm concentration, motility and viability (plasma membrane integrity) were determined. There were no differences in post-thaw sperm concentration, motility or viability but differences in progressive motility between lines were observed (Table 1).

Table 1. Characteristics of frozen-thawed rooster sperm samples.

Line	Concentration (10^6)	Motility (%)	Progressive motility (%)	Viability (%)[1]
1	290.3 ± 73	58 ± 7.6	31 ± 3.1[a]	53 ± 7
2	491.0 ± 80.2	31 ± 8.4	11 ± 3.4[c]	57 ± 8
3	478.3 ± 80.2	22 ± 8.4	12 ± 3.4[c]	34 ± 8
4	453.6 ± 80.2	40 ± 8.4	21 ± 3.4[bc]	40 ± 8
5	575.7 ± 73.0	55 ± 7.6	21 ± 3.1[bc]	39 ± 7
6	581.0 ± 104.5	57 ± 10.4	32 ± 4.4[a]	49 ± 10

[1]Viability is defined as plasma membrane integrity
[abcd] Indicates a significant difference within a column

Interpretations and Recommendations: These results demonstrate that we are freezing rooster sperm repeatedly at a high quality as indicated by the levels of motility, progressive motility and viability. Furthermore, the range of sperm concentrations measured indicates that we are freezing 1.5 to 2.9 inseminations per straw assuming a 100 x 10^6 sperm insemination dose. This research serves as a measure of quality control for our rooster sperm cryopreservation procedures and the information derived from the quantity of insemination doses per straw can be used for better estimating the number of semen straws needed to meet repository requirements.

- P. Purdy
- H. Blackburn

INVESTIGATIONS IN SPERM PHYSIOLOGY, FERTILITY AND IMPROVED CRYOPRESERVATION METHODS

Problem: Fertility can be achieved with cryopreserved sperm but effects of cryopreservation on sperm physiology and fertility are not completely understood. The presence of ubiquitin on the plasma membrane is an indicator of low fertility in sperm but it is not understood how ubiquitin affects sperm physiology or the cryopreservation process. Therefore, research has focused on the role of ubiquitin in sperm physiology and cryopreservation.

Findings: Ubiquitin is a small protein the body places on the surface of defective cells as part of the process of apoptosis (programmed cell death). Reports have documented the presence of ubiquitin on the plasma membrane of spermatozoa and it is theorized that the labeling of sperm occurs for the same rationale. What has not been reported is the influence ubiquitin has on sperm physiology and cryopreservation. Fresh and frozen-thawed boar sperm were analyzed for motility, plasma membrane integrity, acrosomal integrity, membrane fluidity, mitochondrial activity and the presence of ubiquitin. Contrary to other reports, it was found that greater mitochondrial activity is observed in sperm with higher levels of ubiquitin post-thaw but if a sperm loses a large amount of its ubiquitin during the cryopreservation process then it will be of a lower quality post-thaw.

Interpretations and Recommendations: These results demonstrate that ubiquitination affects sperm physiology (mitochondrial activity) and that this may be an indicator of quality following cryopreservation. Investigation of the role of ubiquitin in sperm physiology and cryopreservation is continuing with boars.

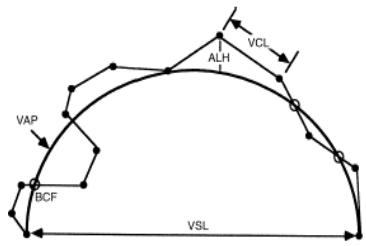
- P. Purdy

VARIATION AMONG COMMERCIAL LINES OF BOARS FOR POST-THAW SEMEN QUALITY

Problem: It has been speculated that there are significant genetic differences between populations of boars for post-thaw semen quality. Such differences have been found between mouse strains. Understanding if there is a genetic basis for this variation is necessary to avoid biases in population sampling that could result in loss of genetic variation.

Findings: Semen samples were collected from 163 boars representing four closed composite lines that are commercially available to US swine producers. The boars were housed at two different commercial boar studs. Two semen samples from each boar were collected at least one week apart. Prefreeze and post thaw CASA analyses were performed on a total of 1,000 sperm cells from at least five fields. Significant sources of variation in sperm cell traits were associated with the stud where the boar was housed, fresh vs post-thaw semen status and among boars. With few exceptions, variation among lines and between collections was not significant and when significance occurred there was no consistent ranking between lines.

Definition of CASA parameters



VAP (mm/sec) -Average path velocity
VCL (mm/sec) - Critical velocity
VSL (mm/sec) - Straight line velocity
BCF (Hz) - Beat cross frequency
STR (VSL/VAP) - Straightness
LIN (VSL/VCL) - Linearity of motion

Line differences for various motion parameters.

Line	VCL	BCF	Progressive motility
1	73.3[ab]	121.0[a]	37.4
2	75.5[b]	113.7[b]	39.2
3	70.7 [ab]	115.4[b]	38.6
7	66.9[a]	116.4[ab]	32.3

Interpretations and Recommendations: These results suggest that line differences for cryosurvival are a minor source of variation and therefore eliminate the need for line specific cryopreservation protocols.

- H. Blackburn
- T. Stewart (Purdue Univ)
- P. Purdy
- F. Gunsett (Newsham Genetics)

PUBLICATIONS FOR NAGP

1. Blackburn, H.D., Welsh, C.S., Stewart, T. 2005. Within breed selection of boars for a gene bank. Journal of Animal Science. 83(Supl 1):138.

2. Purdy, P.H. 2005. A review on goat sperm cryopreservation. Small Ruminant Research. 2005. Published online at: http://www.sciencedirect.com/science/journal/09214488.

3. Purdy, P.H., Fox, M.H., Graham, J.K. 2005. The fluidity of chinese hamster ovary cell and bull sperm membranes after cholesterol addition. Cryobiology. 51:102-112.

4. Purdy, P.H. 2005. The post-thaw quality of ram sperm held for 0 to 48 hours at 5°c prior to cryopreservation. Animal Reproduction Sciences. 2005. Published online at: www.sciencedirect.com/science/journal/03784320.

5. Barrera-Compean, M.H., Purdy, P.H., Dzakuma, J.M., Newton, G.R., Nuti, L.C. 2005. Cholesterol-loaded cyclodextrin improves post-thaw goat sperm motility. Journal of Animal Science. 2005. 83 (Supl 1):153.

6. Cleveland, M.A., Blackburn, H.D., Enns, R.M., Garrick, D.J. 2005. Changes in inbreeding of U.S. herefords during the twentieth century. Journal of Animal Science. 83:992-1001.

7. Purdy, P.H., Stobart, R., Murdoch, W.J., Moss, G.E., Blackburn, H.D. 2005. The fertility of ram sperm held for 24 hours at 5ºc prior to cryopreservation. Journal of Animal Science. (Accepted).

8. Barrera-Compean, M.H., Dzakuma, J.M., Newton, G.R., Nuti, L.C., Purdy, P.H. 2005. Cholesterol-loaded Cyclodextrin treatment is beneficial for goat sperm during cryopreservation. (Accepted).

9. H. D. Blackburn. 2005. The National Animal Germplasm Program: Challenges and Opportunities. Poultry Science. (Accepted).

10. Moce, E., Purdy, P.H., Graham, J.K. 2005. Ram Sperm Cryosurvival Increases after treatment with cholesterol-loaded cyclodextrins. Journal of Andrology. (Accepted).