ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149188 Year: 98 Project Number: 1265-31000-060-00 D
Mode Code: 1265-10-00 STP Codes: 3.1.1.2 50% 3.1.3.1 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: SEX PRESELECTION IN MAMMALS AND SPERM PRESERVATION IN LIVESTOCK AND POULTRY

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

A major problem in the turkey and swine industries is the need for improved utilization and storage of sperm to increase reproductive efficiency and genetic improvement. The problems being specifically addressed are the following: 1) develop better methods to utilize cryopreserved sperm for standard market animal production; 2) determine how competitive fertilization influences paternity efficiency of sperm pooled from large numbers of males; 3) develop technology to preserve the fertility of turkey sperm for more than 6 hours after collection; and 4) improve the method for sorting sperm by sex so that sexed sperm can be used for conventional artificial insemination in swine; 5) develop a laboratory technique to predict the fertilizing potential of sperm from individual males.

2. How serious is the problem? Why does it matter?

During the last 5 years in the U.S.A., the use of artificial insemination in swine has made tremendous strides with the percentage of all market hogs produced by artificial insemination increasing from 5 to 50%. There is a demand for greater efficiency of production, to meet future needs. Improving efficiency will require the widespread use of cryopreserved semen in conjunction with sex preselection. This new technology will enhance the rate of genetic improvement in reproductive and production traits which will reduce the cost of pork production. Artificial insemination using semen pooled from many sires is used exclusively to produce commercial turkeys. This practice is very labor intensive and inefficient in its selection of sires. Genetic improvement of reproductive and production traits is limited in the turkey industry by the absence of techniques for

storage of sperm in liquid or frozen form. Long-term liquid semen storage would be a tremendous benefit to management of males and females at different locations, and to shipment and storage of valuable germplasm. Sire selection has not been a major component of turkey reproduction management. This is because there has been no test to determine the effects of sperm competition on the fertility of sperm from individual males in pooled semen samples. Improved sire selection could have a major impact on the industry savings of millions of dollars annually by eliminating unproductive males. Characterization of individual males used for cryopreserved swine semen production would increase substantially the

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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

savings to the swine industry. This has become doubly important now with the advent of frozen sexed boar semen.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program 101 entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of reproductive efficiency that relate to the problems investigated in this CRIS project, specifically development of improved methods of liquid preservation and cryopreservation of sperm in the pig and the turkey. In addition, this program specifically emphasizes our ongoing research in sex preselection in the mammal.

4. What was your most significant accomplishment this past year?

The most significant accomplishment of the last year in turkey sperm research has been the development and technology transfer of a sperm assessment assay which is highly predictive of fertility. This sire selection test quantitates the differences in sperm motility parameters between males and we have demonstrated its influence on paternity efficiency. Tests have been conducted on primary turkey breeder flocks at two commercial farms. The most significant advance in sperm sexing research has been the increase of sexed sperm production using high speed sorting and a newly developed and unique orienting nozzle. This has increased the production of sexed sperm from .5 million per hour to 5 to 6 million per hour. Using this improved technology, calves of the predicted sex were born from cows inseminated by conventional means. In swine, two studies were conducted that showed a 97% accuracy rate for females born from the use of female producing sperm, while controls averaged 48% females. These studies also demonstrated the effective utilization of in vitro fertilization techniques in conjunction with the sexing technology.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

This project resulted in the invention of the Beltsville Sperm Sexing Method for preselecting sex in mammals. This discovery is considered a major advance in reproductive biology. Subsequent development has resulted in its usefulness for sex preselection in cattle, swine, rabbits, horses, sheep and humans. A recent accomplishment in sexing research has been the development of a new nozzle to orient sperm as they pass the laser beam and its adaptation to a high speed cell sorter. This improved system was used

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Mode Code: 1265-10-00 STP Codes: 3.1.1.2 50% 3.1.3.1 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

to produce female litters of pigs (97% of the correct sex) by surgical transfer of embryos produced with sexed sperm, and the production of female calves from sexed bull semen after conventional artificial insemination. We can sex semen with greater than 90% accuracy at rates exceeding 5 million sperm per hour. This is being adopted by the cattle industry at the present time and will likely reach the commercial market in about a year. The Beltsville Sperm Sexing Technology is also being used by the Genetics and IVF Institute of Fairfax, VA for sex preselection in humans so that families can avoid sex-linked disease. Results were recently published showing a 93% accuracy of producing female children from sexed sperm. We continue to make modifications on the sperm mobility test for classifying and believe it will be an important tool for enhancing the efficiency of turkey sire management. We have made considerable progress in the understanding of paternity efficiency/competitive fertilization when semen is pooled from large numbers of males. Results show that only a few males are producing a large number of offspring which contradicts the assumption that all males are equally contributing to the gene pool. This discovery has resulted in one primary breeder altering their insemination procedures. We expect that this research will alter the way commercial breeders manage there sires. We have also made progress in developing methods for long term storage of semen. Utilizing dehydrin proteins isolated from wheat by ARS scientists, we have demonstrated improved fertility after 24 hour storage of semen at refrigeration temperatures.

6. What do you expect to accomplish during the next year?

We plan to bring together the work we have done with the sperm mobility test in with the paternity efficiency research and demonstrate that turkey males with highly mobile sperm are producing the most offspring in competitive fertilization trials. These results will provide evidence of the importance of evaluating current methods for artificial insemination. With respect to swine we plan to utilize our improved sexing method to demonstrate the feasibility of cryopreserved sexed semen providing a wider application of the process for the swine and cattle industry. In addition we will continue studies to improve the boar semen freezing procedure so it

can be used for commercial market hog production. These studies will involve paying particular attention to membrane changes in frozen semen.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

For turkeys, descriptions and demonstrations of the procedures for sire selection by sperm mobility have been presented at international meetings

Accession: 0149188 Year: 98 Project Number: 1265-31000-060-00 D
Mode Code: 1265-10-00 STP Codes: 3.1.1.2 50% 3.1.3.1 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

and in short courses. Protocols and materials have been distributed to many university scientists and research directors of commercial farms. The Beltsville Sperm Sexing Technology is being transferred to the animal industry (with special emphasis on cattle) by licensure, to Mastercalf, Ltd. of Cambridge, UK and XY, Inc. of Fort Collins, CO. A commercial product is expected within the next year. The use of human sexed sperm will increase, all as the result of the successful clinical trials reported during the past year by the licensee, Genetics and IVF Institute, Fairfax, VA. Protocols and hardware have been transferred to collaborators and interested parties to increase research on the technology on an international scale. The primary constraints are that as a complex technology for sexing semen, it will take time and significant funding to establish commercial usage.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The three most important publications/presentations of this Cris project over the past year are: (1) The increase in the rate of sexed sperm production by the Beltsville Sperm Sexing Technology by 15 fold (from 350 thousand to 5 to 6 million sperm/hour) (2) The development of a method to effectively measure the paternity efficiency of turkey sires through correlation of sperm mobility, fertilizing capability of the sperm and the genetic makeup of individual sires and (3) the birth of 17 litters of pigs from the transfer of embryos produced from sexed sperm with 97% of the offspring being of the predicted sex and use of sexed sperm with conventional artificial insemination in cattle.

PUBLICATIONS:

01.
ABEYDEERA, L.R., JOHNSON, L.A., WELCH, G.R., WANG, W.H., BOQUEST, A.C., CANTLEY, T.C., RIEKE, A. and DAY, B.N. 1998. Birth of piglets preselected for gender following in vitro ... flow cytometry. Theriogenology 49(1):360.

02.
DONOGHUE, A.M., BAKST, M.R., DRUMMOND, P., HAQQUE, S. and SMITH, E.J. 1998.
Paternity efficiency in turkeys differs extensively after heterospermic insemination. Proceedings 8th Internat. Symp. Spermatology 8:62.

03.
DONOGHUE, A.M., HOLSBERGER, D.R., EVENSON, D. and FROMAN, D.P. 1998. Semen
donor selection by in vitro sperm mobility increases fertility and semen storage in the turkey hen. J. Androl. 19:295-301.

04.
DONOGHUE, A.M. and SIMMONS, M.K. 1998. Fertility is improved after supplementing wheat seed dehydration- induced proteins to turkey semen stored 24 hours in vitro. J. Androl. 19:51.

Publications: (Continued)

05.
GURNSEY, M.P. and JOHNSON, L.A. 1998. Recent improvements in efficiency of flow cytometric sorting of X and Y-chromosome bearing sperm of domestic animals: A review. New Zealand Society of Animal Reproduction. 58:42-47.

06.
HOLSBERGER, D.R. 1998. Assessment of ejaculate quality and sperm characteristics in turkeys: Sperm mobility phenotype is independent of time. Thesis. Univ. Maryland. 73 pp.

07.
HOLSBERGER, D.R., DONOGHUE, A.M., FROMAN, D.P. and OTTINGER, M.A. 1998. Assessment of ejaculate quality and sperm characteristics in Turkeys: Sperm mobility phenotype is independent of time. Poult. Sci. 77:1711-1717.

08.
JOHNSON, L.A. 1997. Advances in gender preselection in swine. J. Reprod. Fertil. (Suppl. 52):255-266.

09.
JOHNSON, L.A. 1998. Successful gender preselection in farm animals, Ch. 19, pp. 439-452. IN: A. Altman (ed.) Agricultural Technology. Marcel Dekker, Inc., New York, NY.

10.
JOHNSON, L.A. 1998. Current developments in swine semen: Preservation, artificial insemination and sperm sexing. Proc. of the 15th IPVS Congress. 225-229, Birmingham, England.

11.
JOHNSON, L.A., WELCH, G.R., et al. 1998. High speed sorting of spermatozoa: Procedural adaptations and effects of higher system pressure for enhanced sexing of mammalian sperm based on DNA. Cytometry (Suppl. 9):130.

12.
JOHNSON, L.A., WELCH, G.R., RENS, W. and DOBRINSKY, J.R. 1998. Enhanced flow cytometry sorting of mammalian X and Y sperm: High speed sorting and orienting nozzle for artificial insemination. Theriogenology 49(1):361.

13.
KAWARASAKI, T.,WELCH, G.R., LONG, C.R., YOSHIDA, M. and JOHNSON, L.A. 1998. Verification of flow cytometrically-sorted X- and Y-bearing porcine spermatozoa and reanalysis ... (FISH) technique. Theriogenology 50:625-635.

14.
KING, L.A. and DONOGHUE, A.M. 1998. Computer Aided (CASA) measurement of turkey sperm mobility parameters: Correlation with a sire selection test. Poult. Sci. 77:90.

15.
LONG, C.R., RATH, D., WELCH, G.R., SCHREIER, L.L., DOBRINSKY, J.R. and JOHNSON, L.A. 1998. In vitro production of porcine embryos from semen sorted for sex with a high speed cell ... media. Theriogenology 49(1):363.

Publications: (Continued)

16.
MAXWELL, W.M.C. and JOHNSON, L.A. 1997. Membrane status of boar spermatozoa after cooling or cryopreservation. Theriogenology 48:209-219.

17.
PENFOLD, L.M.,HOLT, C., HOLT, W.V., WELCH, G.R., CRAN, D.G. and JOHNSON, L.A. 1998. Comparative motility of X and Y chromosome-bearing bovine sperm separated on the basis of ... flow sorting. Mol. Reprod. Dev. 50:323-327.

18.
RENS, W., WELCH, G.R. and JOHNSON, L.A. 1998. Flow cytometric sperm sorting: A novel nozzle that increases sperm orientation and sorting efficiency. Cytometry (Suppl. 9):131.

19.
RENS, W., WELCH, G.R., JOHNSON, L.A. 1998. A novel nozzle for more efficient sperm orientation to improve sorting efficiency of X and Y chromosome-bearing sperm. Cytometry 33:476-481.

20.
WELCH, G.R., RENS, W. and JOHNSON, L.A. 1998. High speed cell sorting: Modifications to a Moflo for sorting X and Y chromosome bearing sperm based on DNA. Cytometry (Suppl. 9):130.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401417 Year: 98 Project Number: 1265-31000-060-03 S
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: ESTABLISHING A SIRE-SELECTION COMPONENT FOR TURKEY BREEDER MANAGEMENT

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

A major problem in the turkey industry is the need for improved utilization of sperm to increase reproductive efficiency and genetic improvement. Two specific objectives of this CRIS are the following: 1) develop a laboratory technique to predict the fertilizing potential of sperm from individual males and 2) determine how competitive fertilization influences paternity efficiency of sperm pooled from large number of males.

2. How serious is the problem? Why does it matter?

Artificial insemination is used exclusively to reproduce commercial turkeys making this practice very labor intensive. Unlike other agriculturally important species, sire selection has not been a component of turkey reproduction. This in combination of with the paucity of information on the effects on sperm competition in commercial production has resulted in a very inefficient use of potential sires. Sire selection could have a major impact on the industry savings of millions of dollars annually by eliminating males not contributing to offspring production and the associated costs of feed and labor.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program 101 entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of

reproductive efficiency that relate to the problems investigated in this CRIS project, specifically development of improved methods of liquid preservation and cryopreservation of sperm in the pig and the turkey. In addition, this program specifically emphasizes our ongoing research in sex preselection in the mammal.

4. What was your most significant accomplishment this past year?

The most significant accomplishment of the last year has been the development and technology transfer of a sperm assessment assay which is

Accession: 0401417 Year: 98 Project Number: 1265-31000-060-03 S
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

highly predictive of fertility. This sire selection test quantitates the differences in sperm motility parameters between males and we have demonstrated its influence on paternity efficiency.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We continue to make modifications on the sperm mobility test and believe it will be an important tool for enhancing the efficiency of male management. We have made considerable progress in the understanding of paternity efficiency/competitive fertilization when semen is pooled from large numbers of males. Our results have demonstrated that only a few males are producing a large number of offspring which contradicts the assumption that all males producing white, highly concentrated clean ejaculates are equally contributing to the gene pool. This discovery has resulted in one primary breeder altering their insemination procedures. We expect that this research will alter the way commercial breeders manage there sires.

6. What do you expect to accomplish during the next year?

We plan to bring together the work we have done with the sperm mobility test with the paternity efficiency research and demonstrate that males with highly mobile sperm are producing the most offspring in competitive fertilization trials. These results will provide evidence of the importance of evaluating current methods for artificial insemination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Descriptions and demonstrations of the procedures for sire selection by sperm mobility have been presented at international meetings and in short courses. Protocols and materials have been distributed to many university scientists and research directors of commercial farms.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The most important presentation of this CRIS project was Computer Aided (CASA) measurement of turkey sperm mobility parameters: Correlation with a sire selection test.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401318 Year: 98 Project Number: 1265-31000-060-04 T
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: ASSESSMENT OF SPERM MOTILITY: ESTABLISHMENT OF A SIRE- SELECTION COMPONENT FOR TURKEY BREEDER MGMT

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

A major problem in the turkey industry is the need for improved utilization of sperm to increase reproductive efficiency and genetic improvement. Two specific objectives of this CRIS are the following: 1) develop a laboratory technique to predict the fertilizing potential of sperm from individual males and 2) determine how competitive fertilization influences paternity efficiency of sperm pooled from large number of males.

2. How serious is the problem? Why does it matter?

Artificial insemination is used exclusively to reproduce commercial turkeys making this practice very labor intensive. Unlike other agriculturally important species, sire selection has not been a component of turkey reproduction. This in combination of with the paucity of information on the effects on sperm competition in commercial production has resulted in a very inefficient use of potential sires. Sire selection could have a major impact on the industry savings of millions of dollars annually by eliminating males not contributing to offspring production and the associated costs of feed and labor.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program 101 entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of

reproductive efficiency that relate to the problems investigated in this CRIS project, specifically development of improved methods of liquid preservation and cryopreservation of sperm in the pig and the turkey. In addition, this program specifically emphasizes our ongoing research in sex preselection in the mammal.

4. What was your most significant accomplishment this past year?

The most significant accomplishment of the last year has been the testing of this procedure in field trials on primary turkey breeder flocks at two

Accession: 0401318 Year: 98 Project Number: 1265-31000-060-04 T
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

commercial farms.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We continue to make modifications on the sperm mobility test and believe it will be an important tool for enhancing the efficiency of male management. We have made considerable progress in the understanding of paternity efficiency/competitive fertilization when semen is pooled from large numbers of males. Our results have demonstrated that only a few males are producing a large number of offspring which contradicts the assumption that all males producing white, highly concentrated clean ejaculates are equally contributing to the gene pool. This discovery has resulted in one primary breeder altering their insemination procedures. We expect that this research will alter the way commercial breeders manage there sires.

6. What do you expect to accomplish during the next year?

We plan to bring together the work we have done with the sperm mobility test with the paternity efficiency research and demonstrate that males with highly mobile sperm are producing the most offspring in competitive fertilization trials. These results will provide evidence of the importance of evaluating current methods for artificial insemination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Descriptions and demonstrations of the procedures for sire selection by sperm mobility have been presented at international meetings and in short courses. Protocols and materials have been distributed to many university scientists and research directors of commercial farms.

8. List your most important publications and presentations, and articles

written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The most important presentation of this CRIS project was entitled Assessing potential fertility of individual males: Evaluation of sperm characteristics and function in turkeys.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401533 Year: 98 Project Number: 1265-31000-060-05 S
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: ESTABLISHING A SIRE-SELECTION COMPONENT FOR TURKEY BREEDER MANAGEMENT

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

A major problem in the turkey industry is the need for improved utilization of sperm to increase reproductive efficiency and genetic improvement. Two specific objectives of this CRIS are the following: 1) develop a laboratory technique to predict the fertilizing potential of sperm from individual males and 2) determine how competitive fertilization influences paternity efficiency of sperm pooled from large number of males.

2. How serious is the problem? Why does it matter?

Artificial insemination is used exclusively to reproduce commercial turkeys making this practice very labor intensive. Unlike other agriculturally important species, sire selection has not been a component of turkey reproduction. This in combination of with the paucity of information on the effects on sperm competition in commercial production has resulted in a very inefficient use of potential sires. Sire selection could have a major impact on the industry savings of millions of dollars annually by eliminating males not contributing to offspring production and the associated costs of feed and labor.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program 101 entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of

reproductive efficiency that relate to the problems investigated in this CRIS project, specifically development of improved methods of liquid preservation and cryopreservation of sperm in the pig and the turkey. In addition, this program specifically emphasizes our ongoing research in sex preselection in the mammal.

4. What was your most significant accomplishment this past year?

The most significant accomplishment of the last year has been the testing of this procedure in field trials on primary turkey breeder flocks at two

Accession: 0401533 Year: 98 Project Number: 1265-31000-060-05 S
Mode Code: 1265-10-00 STP Codes: 3.1.3.1 50% 3.1.3.2 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

commercial farms.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We continue to make modifications on the sperm mobility test and believe it will be an important tool for enhancing the efficiency of male management. We have made considerable progress in the understanding of paternity efficiency/competitive fertilization when semen is pooled from large numbers of males. Our results have demonstrated that only a few males are producing a large number of offspring which contradicts the assumption that all males producing white, highly concentrated clean ejaculates are equally contributing to the gene pool. This discovery has resulted in one primary breeder altering their insemination procedures. We expect that this research will alter the way commercial breeders manage there sires.

6. What do you expect to accomplish during the next year?

We plan to bring together the work we have done with the sperm mobility test with the paternity efficiency research and demonstrate that males with highly mobile sperm are producing the most offspring in competitive fertilization trials. These results will provide evidence of the importance of evaluating current methods for artificial insemination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Descriptions and demonstrations of the procedures for sire selection by sperm mobility have been presented at international meetings and in short courses. Protocols and materials have been distributed to many university scientists and research directors of commercial farms.

8. List your most important publications and presentations, and articles

written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The most important presentation of this CRIS project was entitled Assessing potential fertility of individual males: Evaluation of sperm characteristics and function in turkeys.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR
OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401708 Year: 98 Project Number: 1265-31000-060-06 T
Mode Code: 1265-10-00 STP Codes: 3.1.1.2 50% 3.1.3.1 50%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: ASSESSMENT OF QUALITY OF POULTRY SPERM

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

A major problem in the turkey industry is the need for improved utilization of sperm to increase reproductive efficiency and genetic improvement. The specific objective of this CRIS project is develop a laboratory technique to predict the fertilizing potential of sperm from individual males.

2. How serious is the problem? Why does it matter?

Artificial insemination is used exclusively to reproduce commercial turkeys making this practice very labor intensive. Unlike other agriculturally important species, sire selection has not been a component of turkey reproduction. Sire selection could have a major impact on the industry savings of millions of dollars annually by eliminating males not contributing to offspring production and the associated costs of feed and labor.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program 101 entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of reproductive efficiency that relate to the problems investigated in this CRIS project, specifically development of improved methods of liquid preservation and cryopreservation of sperm in the pig and the turkey. In addition, this program specifically emphasizes our ongoing research in sex

preselection in the mammal.

4. What was your most significant accomplishment this past year?

The most significant accomplishment of the last year has been the development and technology transfer of a sperm-egg binding assay which is predictive of fertility. This sire selection test quantitates the differences in sperm binding ability between males. We have tested this procedure in fertility trials.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

We continue to make modifications on the sperm binding test and believe it will be an important tool for enhancing the efficiency of male management.

6. What do you expect to accomplish during the next year?

We plan to bring together the work we have done with the sperm binding test with the paternity efficiency research and demonstrate that males with highly binding sperm are producing the most offspring in competitive fertilization trials. These results will provide evidence of the importance of evaluating current methods for artificial insemination.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

Descriptions and demonstrations of the procedures for this work has been presented at national meetings.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

An important presentation for this CRIS project was given by Dr. Donoghue at National Breeders Round Table "Assessing potential fertility of individual males: Evaluation of sperm characteristics and function in turkeys".

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149987 Year: 98 Project Number: 1265-31000-065-00 D
Mode Code: 1265-10-00 STP Codes: 3.1.2.1 10% 3.1.3.2 90%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: USDA ANIM HORMONE PROGRAM & THE FUNCTION OF GENES REGULATING TURKEY EGG PRODUCTION & EMBRYOGENESIS

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Two major problems in the poultry industry are: 1)turkey breeder hens have the poorest hatching egg production of any commercial species of poultry and 2) nearly 20% (about 100 million eggs) of the turkey hatching eggs placed in incubators fail to hatch. The specific objectives being addressing are: 1)determining the molecular basis for the premature cessation of egg production and onset of incubation behavior in turkey hens; 2) developing means to readily differentiate fertilized from unfertilized ova and early dead embryos; 3) determining the molecular and cellular mechanism of early embryonic death; 4) determine the impact of egg storage on the subsequent viability and development of the embryo through incubation, growth and maturation.

2. How serious is the problem? Why does it matter?

Discussions with industry leaders show that incubation behavior is among the biggest problems of the turkey breeder industry because a substantial loss of egg production (>10 eggs/hen even under good management conditions), and because high labor costs are associated with the management necessary to control this behavior. A study conducted under commercial conditions indicated that more than 1% of hens were identified daily as incubating, and more than 50% of farm labor costs were spent on prevention or disruption of this behavior. Improvement in average egg production by 10 eggs/hen would save the industry about $30 million/year. The losses to the industry due to failure of 100 million eggs to hatch can be measured in the cost of producing those eggs (about $60 million) and losses in potential sales revenue. The basis for these losses are both

genetic (selection for heavier turkeys has a negative impact on reproductive efficiency) and management (cold storage of eggs has a negative impact on hatchability). As the market demands heavier birds to meet the demand for further processed turkey products, egg production and hatchability are expected to continue to slowly decline. Furthermore, as the bird becomes genetically predisposed to embryonic mortality, egg storage will further exacerbate egg losses. There is a need to address this problem based on biological data and not solely on empirical observations at the hatchery.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of reproductive efficiency that relate to the problems investigated in this CRIS project.

4. What was your most significant accomplishment this past year?

The most significant accomplishment in understanding incubation behavior was the finding that cellular changes in the pituitary gland at the onset of incubation appear to occur primarily through the conversion of one cell type (somatotrophs) to a different function (lactotrophs), while the reversal of pituitary function at the end of incubation occurs through the programmed death of lactotrophs and appearance of new somatotrophs. The most significant accomplishment in the study of hatchability was the finding that during cold (18 degrees C) egg storage, a routine practice in the turkey industry, early embryogenesis in the turkey is not totally suppressed in all embryos. Although few embryos advanced developmentally at 15C, embryogenesis progressed developmentally with each increment in temperature (20, 25, 30 degrees C). Previously, investigators claimed that "physiological zero", which is the temperature producing developmental arrest in the embryo, was between 20 and 27 degrees C. Our work dispels this notion. Coupled with our earlier observations that the embryonic cell number decreases by 30-40% with egg storage, the following working hypothesis on the biological basis of embryonic cell death was proposed and will be basis of future work: for normal embryonic development to be initiated at incubation, the embryo must be in a certain stage of development (pre-gastrula stage), and possess a yet to be determined minimum number of viable blastodermal cells; if one or both these conditions are not met the embryo will fail to develop normally and possibly die during incubation. Collateral work on the mechanism of prolonged oviductal sperm storage has resulted in the discovery that the sperm-storage tubule, the anatomical unit which actually stores sperm, is innervated. The most significant accomplishment for the USDA-ARS Animal Hormone Program has been the development of monoclonal antibodies which specifically bind to chicken follicle stimulating hormone (FSH). Use of

these antibodies has shown that the chicken has two distinct types of gonadotrophs: those containing only FSH and those containing only luteinizing hormone (LH). Previous research has shown that these two hormones are produced within the same pituitary cells in all species (ranging from humans to frogs) studied to date except the bovine, where LH and FSH also reside in separate cells. Since these hormones function independently during reproduction, a major unanswered question in reproductive biology is how the production and secretion of these hormones is independently regulated. The antibodies produced for the Animal Hormone Program will permit studies on the role of FSH in regulating egg

Accession: 0149987 Year: 98 Project Number: 1265-31000-065-00 D
Mode Code: 1265-10-00 STP Codes: 3.1.2.1 10% 3.1.3.2 90%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

production.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

The major accomplishments over the life of this project that are related to hatchability include: 1) development of a objective staging procedure describing the morphological sequence of embryogenesis in the turkey and duck (this procedure is currently being used by other investigators addressing aspects of early embryonic development); 2) provided observations and guidelines to visually differentiate fertilized from unfertilized ova and to identify early dead turkey embryo (this knowledge is being used by some commercial turkey, chicken and duck breeders to better understand the basis of hatch losses whether it is due to a fertility problem or an embryo mortality problem); and 3) developed procedures for the isolation and culture of immature primary follicular oocytes from turkeys. Major accomplishments related to improving the efficiency of egg production include: 1) finding that pituitary lactotrophs increase dramatically in both number and size during the transition from egg laying to incubation behavior, resulting increased prolactin secretion and ovarian regression; 2) demonstrating that the termination of incubation behavior results in the programmed death of lactotrophs and their replacement with somatotrophs through unknown mechanisms; 3) identification of bi-hormonal mammosomatotrophs in turkey pituitaries, the first such finding in an avian species, and demonstration that an increase in the proportion of mammosomatotrophs cells is associated with incubation behavior; and 4) demonstration that FSH and LH are produced in separate gonadotrophs in the chicken.

6. What do you expect to accomplish during the next year?

In the next year we plan to investigate the impact of egg storage on embryonic cell proliferation capabilities and the expression of specific genes at a specific stages of development. The impact of long term (30 day) egg storage on the development of the embryonic gut and post-hatch function

will be the basis of a BARD proposal with an Israeli investigator. The impact of long term (30 day) egg storage on the distribution of fibronectin will also be investigated. Studies on the cellular mechanisms which initiate the proliferation or death of lactotrophs in response to environmental or neural signals will continue, focusing on the identification of neuropeptides and neurotransmitters which initiate the programmed death of lactotrophs and proliferation of somatotrophs. New monoclonal antibodies to a novel prolactin-related protein in the turkey and chicken pituitary will be characterized and used to study the role of this protein in reproduction. New non-radioactive assay techniques will be

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Mode Code: 1265-10-00 STP Codes: 3.1.2.1 10% 3.1.3.2 90%
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developed for the measurement of avian prolactin and chicken FSH, and reagents for these techniques will be made available to scientists worldwide through the USDA Animal Hormone Program.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

In the area of embryonic development and hatchability, technology transfer comes in the form of training visiting scientists, technicians and commercial hatchery personnel in the techniques described in Question 5 and in their publication and presentations at national and international meetings and workshops. In the past year, individual(s) from the GGPL staff, North Carolina State University, University of Maryland, Perdue Farms (broiler) and Maple Leaf Farms (duck) were trained to differentiate egg status (fertilized, unfertilized, dead embryo) at the time of lay. Constraints for industry regarding adapting this technology are their lack of time and personnel to devote to learn and then adapt the techniques to their quality control program. An invited presentation at a major international meeting of turkey industry personnel provided a comprehensive explanation of the ways in which environmental and biological factors interact to cause lost egg production through incubation behavior, and how this knowledge may be applied to improve reproductive efficiency. Descriptions of the production and use of monoclonal antibodies to chicken FSH and avian prolactin and a prolactin-related protein were presented at national and international scientific conferences, and the immunohistochemical techniques used to identify two distinct types of avian gonadotrophs have been accepted for publication. The USDA-ARS Animal Hormone Program has provided research reagents to dozens of scientists worldwide, and we have responded to many requests for advice on the use of pituitary hormones or hormone assays in farm animal and biomedical research.

8. List your most important publications and presentations, and articles

written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

The most important presentations of this CRIS were: 1) the ARS Information staff write-up on turkey egg hatchability as it generated considerable public interest; 2) the article on oviductal sperm storage, which generated interest from academics working with fish and amphibians; 3) the symposium presentation to industry representatives on incubation behavior; and 4) demonstration that two distinct populations of gonadotrophs exist in an avian species.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149987 Year: 98 Project Number: 1265-31000-065-00 D
Mode Code: 1265-10-00 STP Codes: 3.1.2.1 10% 3.1.3.2 90%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

PUBLICATIONS:

01.
BAKST, M.R. 1998. Structure of the avian oviduct with emphasis on sperm storage in poultry. J. Exp. Zool. 282:618-626.

02.
BAKST, M.R., GUPTA, S.K ., POTTS, W. and AKUFFO, V. 1998. Gross appearance of the turkey blastoderm at oviposition. Poultry Sci. 77: 1228-1233.

03.
KUENZEL, W.J., RAMESH, R., et al. 1998. Changes in thyrotrophs and gonadotrophs within the pituitary gland during induction of advanced gonadal development by sulfamethazine. Poultry Sci. 77 (Suppl. 1):18.

04.
PROUDMAN, J.A. 1997. Physiological basis of broodiness in turkey breeder hens. Proc. Fourth Int. Symp. Turkey Reproduction: 143-151, North Carolina State University, Raleigh, NC.

05.
PROUDMAN, J.A. 1998. Circulating prolactin levels at the end of the photophase and the end of the scotophase throughout the reproductive cycle of the turkey hen. Poultry Sci. 77:303-308.

06.
PROUDMAN, J.A., VANDESANDE, F. and BERGHMAN, L.R. 1998. Production and characterization of monoclonal antibodies to chicken follicle stimulating hormone. Poultry Sci. 77(Suppl. 1):18.

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RICKES, E.L., CHANG, C.H., McNAMARA, L., NARGUND, R., PROUDMAN, J.A. and HICKEY, G.J. 1998. Growth hormone (GH) secretagogue activity of various

nonpeptidyl GH secretagogues in chicks. J. Anim. Sci. 76(Suppl. 1):122.

08.
YORKS, A., RATTNER, B., et al. 1998. Investigations of potential endocrine disruptions and sexual dimorphism in ... (tachycineta bicolor) with a range of PCB body burdens. Soc. Environ. Toxico. Chem. Abstr. Book, p. 118.

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

OFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401393 Year: 98 Project Number: 1265-31000-065-02 T
Mode Code: 1265-10-00 STP Codes: 3.1.3.2 100%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: BEHAVIOR-INDUCED NEURAL AND NEUROENDOCRINE REGULATION OF PITUITARY CELL DIFFERENTIATION

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Turkeys have the poorest egg production and shortest reproductive cycle of any commercial poultry species. The average commercial turkey hen produces 100 eggs in a 24 week reproductive season, while broiler breeder hens lay 175 eggs over a 40 week season, and egg-type chickens lay 280 eggs in a year. Turkeys have been selected intensively for growth rate, and the negative genetic correlation between body weight and egg production has reduced reproductive efficiency. Turkey hens exhibit a high incidence of incubation behavior, which results in a substantial loss of egg production. New immunopharmaceutical techniques are being developed to improve reproductive efficiency by reducing incubation behavior. This Agreement assists in assessing the effectiveness of these techniques.

2. How serious is the problem? Why does it matter?

Discussions with industry leaders show that incubation behavior is among the biggest problems of the turkey breeder industry because a substantial loss of egg production (>10 eggs/hen even under good management conditions), and because high labor costs are associated with the management necessary to control this behavior. A study conducted under commercial conditions indicated that more than 1% of hens were identified daily as incubating, and more than 50% of farm labor costs were spent on prevention or disruption of this behavior. Improvement in average egg production by 10 eggs/hen would save the industry about $30 million/year.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This Agreement was implemented to help solve a problem outlined in the National Research Program entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the area of improvement of reproductive efficiency, which relates to the investigations covered by this Agreement.

4. What was your most significant accomplishment this past year?

The use of assay technology developed in this laboratory has permitted the

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evaluation of the effectiveness of various immunopharmaceutical techniques for controlling plasma prolactin levels in turkey hens.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

See Question 4. These findings, combined with data collected elsewhere, will lead to development of new technology that will improve the efficiency of hatching egg production in the turkey.

6. What do you expect to accomplish during the next year?

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption durability of the technology?

The patented technologies under development by the Cooperator require field trials and FDA approval before they will be available to the industry. These trials are underway.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

No publications.

PUBLICATIONS:

Approved: D. F. COLE Date: 02/99
Title: ACTING ASSOCIATE DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401542 Year: 98 Project Number: 1265-31000-065-03 S
Mode Code: 1265-10-00 STP Codes: 3.1.3.2 100%
NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

Title: BEHAVIOR-INDUCED NEURAL AND NEUROENDOCRINE REGULATION OF PITUITARY CELL DIFFERENTIATION

Period Covered From: 01/98 To: 12/98

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Commercial turkey breeder hens have the poorest reproductive efficiency of any avian species. One of the reasons for the low productivity is expression of incubation behavior or broodiness by the hens at their peak of egg laying. This project investigates the mechanisms underlying the differentiation of adult pituitary cells associated with dramatic changes in pituitary function which occur during the onset and termination of incubation behavior. An understanding of the mechanisms involved should show why some hens exhibit this behavior while others do not, and enable us to devise strategies for elimination of the behavior through genetic selection or management changes.

2. How serious is the problem? Why does it matter?

Discussions with industry leaders show that incubation behavior is among the biggest problems of the turkey breeder industry because a substantial loss of egg production (>10 eggs/hen even under good management conditions), and because high labor costs are associated with the management necessary to control this behavior. A study conducted under commercial conditions indicated that more than 1% of hens were identified daily as incubating, and more than 50% of farm labor costs were spent on prevention or disruption of this behavior. Improvement in average egg production by 10 eggs/hen would save the industry about $30 million/year.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This CRIS project was designed to solve problems outlined in the National Research Program entitled "Animal Germplasm, Resources, Genetics, and Reproduction". This National Research program specifically emphasizes problems in the areas of germplasm preservation and improvement of reproductive efficiency that relate to the problems investigated in this CRIS project.

4. What was your most significant accomplishment this past year?

We have found that the cellular composition of the anterior pituitary gland

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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

shows remarkable morphological changes during the onset and termination of incubation behavior. For instance, lactotrophs replace somatotrophs at the onset of incubation behavior. Such a morphological change is associated with increased secretion of prolactin from the anterior pituitary gland into the blood circulation. Furthermore, we found this change to be associated with an increased abundance of mammosomatotrophs, a cell which contains both prolactin and growth hormone, in the pituitary of incubating hens. This increased abundance of mammosomatotrophs may indicate that conversion of cells from growth hormone production to prolactin production may play a key role in the onset of incubation behavior. In contrast to the onset of incubation behavior, termination of broodiness (by depriving nesting stimuli) results in programmed cellular death of lactotrophs and reappearance of somatotrophs.

5. Describe your major accomplishments over the life of the project, including their predicted or actual impact

Since this is the first annual report, we predict that our findings to date will lead to new research on the cellular mechanisms which cause differentiated cells to change function in response to environmental and behavioral signals.

6. What do you expect to accomplish during the next year?

In the next year we plan to study the time course of the cellular events leading to lactotroph death and somatotroph proliferation in turkey hens that are deprived of nesting stimuli to terminate incubation. These studies may lead to the identification of neuropeptides which cause similar changes in cultured pituitary cells. Neural tract tracing techniques will be developed to aid in identification of brain areas that are stimulated by nesting.

7. What technologies have been transferred and to whom? When is the technology likely to become available to the end user (industry, farmer, other scientists)? What are the constraints, if known, to the adoption

durability of the technology?

Descriptions of the immunohistochemical techniques used to identify mammosomatotrophs in avian pituitaries, and to identify pituitary cells undergoing programmed cell death, have been published, presented at scientific meetings, and sent to interested researchers during the past year.

8. List your most important publications and presentations, and articles written about your work (up to three total--NOTE: this does not replace your reviewed publications which are listed below)

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401542 Year: 98 Project Number: 1265-31000-065-03 S
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NATL PROG(S) 101 Animal Genomes, Germplasm, Reproduction & Development 100%

The most important presentations of this CRIS were: 1)the publication demonstrating, for the first time, the occurrence of mammosomatotrophs in an avian species, and the association of these cells with incubation behavior; and 2) the presentation of results showing that termination of incubation behavior results in programmed cell death of large numbers of pituitary lactotrophs.

PUBLICATIONS:

01.
RAMESH, R., SOLOW, R., PROUDMAN, J.A. and KUENZEL, W.J. 1998. Identification of mammosomatotrophs in the turkey hen pituitary: Increased abundance during hyperprolactinemia. Endocrinology 139: 781-786.

02.
RAMESH, R., KUENZEL, W.J. and PROUDMAN, J.A. 1998. Nest-deprivation of incubating turkey hens is associated with a decline in proliferating cell nuclear antigen in the anterior ... gland. Poult. Sci. 77(Suppl. 1):17.

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