NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Project No. RM2-A12
Date
Submitted .....
Approved .....
Revised .....

1. Title: **Studies of Factors Affecting the Reproductive Processes of Farm Animals.**

2. Objective(s): **(a) To determine whether there are differences among various forage plants, especially alfalfa and grasses, fresh and cured, as sources of nutrients for bulls in reproductive service.**

(b) To determine the effect of sustained general fever during calfhood on the subsequent fertility of dairy sires.

(c) To determine whether there is a positive effect on fertility from the oral administration of synthetic thyroprotein ("Protamone") to dairy cows of low fertility.

(d) In general, to attempt to bring to light some of the factors which: (1) cause differences in fertility rates among various animals of a species, and (2) cause periodic variations in the fertility of individual animals.

3. Reasons for undertaking Investigations*: **It is well known that the maintenance of continuous optimum fertility in our domestic animals is a major problem which faces all practical husbandmen. Large amounts of potential income are lost each year to livestock men in North Carolina as elsewhere as a result of sterility or irregular breeding among the animals they own.**

Further details as to reasons for undertaking the investigations are considered under each subproject heading.

*Including economic justification

SUBPROJECT A

1. Subtitle: A Comparison of Legumes and Grasses, Fresh and Cured, as Sources of Nutrients for Bulls in Breeding Service.

2. Objectives: To determine whether there are differences among various roughages, when fed to bulls, in their effect on the quantity, quality, and fertility of semen produced. More specifically to ascertain whether there is a depressing effect on quality and fertility of semen produced where leafy, green alfalfa hay, or lush pasture, is the sole source of roughage for bulls.

3. Reasons for undertaking investigations: Many breeders in some areas (New York State, for example) refuse to feed high quality alfalfa hay to bulls, because of their belief that bulls do not maintain a high level of fertility when they receive this hay, especially as the sole roughage.

German workers are reported to state that breeding males should not receive large amounts of fresh pasture grass, since this practice may result in lowered fertility.

4. Previous work: The leader of this project, while employed at another institution, conducted a comparison of alfalfa and timothy hays as roughages for bulls in heavy service in artificial insemination (Elliott, F. I., Ph.D. Thesis, Cornell University, 1944). No differences in quantity or quality of semen produced were noted, nor were there differences in fertility. The alfalfa hay used wasleafy green hay, but it had been stored for eight or nine months, so that it was not fresh hay. It is possible that there may be a factor or factors in freshly cured hay, or in fresh pasture, which would have a depressing effect on semen production. This latter seems to be the opinion of some practical dairymen. The problem seems worthy of further investigation, especially in light of present efforts toward increasing the production of high quality hay and pasture on livestock farms in North Carolina.

5. Outline of procedure:

(a) Two groups of sexually mature bulls (numbers to be determined in cooperation with the Institute of Statistics) will be used in the experiment.

(b) During a preliminary period the bulls will all receive the same roughage, preferably a mixed hay, in normal amounts, about 20 pounds or less per bull per day. This period should be at least 2 months in length.

(c) This preliminary period will be followed by the experimental period, of equal length, during which one group will receive alfalfa hay of the highest quality available as the only roughage. The other group will continue to receive the mixed hay. The experimental period should fall during the autumn months.

(d) A post-experimental period will follow, during which all bulls will once more receive the same, or mixed, hay.

(e) Accurate records will be kept of all feed consumed.

(f) The usual chemical analyses as well as carotene will be run on each hay and on the concentrate ration.

(g) Semen will be collected at periodic intervals from each bull, and each sample will be subjected to tests for quality, concentration, motility, percentage of abnormal spermatozoa, etc.

(h) Where possible, the semen will also be used for inseminating normal cows in order to get some information as to the fertility of the samples. Probably it will not be possible to follow any design very closely with respect to this latter factor, since the number of cows available for breeding to any one semen sample will be quite limited.

(i) A similar outline will be followed, previously or subsequently, depending upon feed availabilities, in comparing fresh pasture and cured hay as sources of nutrients for bulls.

(j) If differences among the various feeds, in their effect on reproductive capacity, are found, further studies will be undertaken to determine what specific factors are involved.

(k) Animals used will be located at the College Dairy Farm.

SUBPROJECT B

1. Subtitle: Effect of High Fever During Calfhood on the Subsequent Fertility of Bulls.

2. Objectives: (a) To determine whether sustained general fever, before puberty, results in lowered fertility in bulls, when they become sexually mature.

(b) To further determine whether, if positive results are obtained in (a), ice packs in the scrotal region during the fever period, or other treatment, will prevent the occurrence of lowered fertility.

3. Reasons for undertaking investigations: It is rather well known that general fever in a mature bull results in a subsequent period of lowered fertility. Anderson (The Semen of Animals and Its Use for Artificial Insemination, Imp. Agr. Bureau, 1945) believes that the lowered fertility is a result of the action of increased body temperature on spermatogenesis rather than a direct effect of the actual disease process.

A case of a valuable sire which has had very low fertility throughout life has come to our attention. This bull had a severe infection of hemorrhagic septicemia at four months of age, and the herdsman attributes the low fertility to the infection.

If it can be shown that high or sustained fever during pre-puberty days has a permanent, injurious effect on the fertility of bulls, the next obvious step is to determine whether or not this effect can be alleviated or eliminated by appropriate treatment.

4. Previous work: As far as the author is aware, no work has been done on this problem. All workers in the field realize, however, that one serious difficulty facing those working with artificial insemination is the fact that many bulls are low in fertility from the time they reach puberty, for as yet unexplained reasons. In all probability some of these differences are a result of the environmental conditions under which the bulls were raised. Fever may well be one of the important environmental factors involved.

5. Outline of procedure:

(a) Four to six young bulls of similar age and breeding will be used. All will be fed and handled as nearly alike as possible, except that one-half of the group of animals will be subjected to continuous high fever over a period of two weeks or more, when they are approximately four months of age. The fever will be induced by the administration of Pyrogenic drugs.

(b) The animals will be so fed and handled as to produce maximum growth and development.

(c) Case histories will be kept on all animals, including records of growth rates and body temperature.

(d) When the bulls reach sexual maturity, weekly collections of semen from each bull will be made, and these will be studied in the laboratory to learn what, if any, have been the effects of the fever. Laboratory studies of the semen will include:

(1) Density and volume.

(2) Motility.

(3) Methylene-blue reduction time.

(4) Percentage of abnormal spermatozoa.

(e) If definite effects on reproductive capacity as a result of the fever are discovered, another comparable group of bulls will be placed on experiment, but an ice bag will be placed on the scrotum of each experimental bull during the fever period in order to determine whether this treatment might reduce or negate the effect of the fever on subsequent spermatogenesis.

SUBPROJECT C

1. Subtitle: Effect of Feeding Synthetic Thyroprotein (Protamone) to Dairy Cows of Low Fertility.

2. Objectives: (a) To determine whether Protamone might increase fertility in cows which are functionally sterile.

(b) If the material is effective, to establish proper feeding levels, and further to establish the limits of its usefulness for this purpose.

3. Reasons for undertaking investigations:
   (a) The use of natural thyroxine in cases of sterility in the human female is a common practice of many obstetricians.
   (b) The synthetic thyroid-active hormone, "Protamone," has been used successfully in some cases of sterility in the bull.
   (c) There are many cows which are functionally sterile, that is, they are not diseased, but do not conceive. Some of these cows tend to fatten easily, which may indicate some hypothyroidism.

4. Previous work: As far as the author is aware no previous work has been done on the use of "Protamone" or thyroxine in the treatment of cows which are functionally sterile.

5. Outline of procedure:
   (a) As many "functionally sterile" cows as possible will be placed on the experiment. This will probably involve cows in the station and college herds, as well as sub-station and possibly other herds.
   (b) Insofar as possible, when a cow is placed on treatment another cow with a similar history in the same herd will be used as a control.
   (c) Treatment will continue through several estrous cycles, or until the cow conceives.
   (d) In the beginning, 5 g. of the material will be fed daily to each cow. As more information is obtained, this level may have to be changed.
   (e) Records of body weight, feed intake, and heart rate will be kept on each cow.

6. Probable Duration of Project: Three years. Project will be discontinued or revised and additional subprojects added at that time.

7. Date of Initiation: July 1, 1947.

8. Personnel:

Name	Department	Relation to Project
P. I. Elliott	Animal Industry (Dairy)	Leader
C. D. Grinnells	Animal Industry (Dairy)	Co-leader
R. K. Waugh	Animal Industry (Dairy)	Cooperator
R. H. Ruffner	Animal Industry (Dairy)	Adviser
D. W. Colvard	Animal Industry (Dairy)	Adviser

9. Coöperation:

   a. Interdepartmental - Institute of Statistics.

   b. Other Agencies - Cerophyl Laboratories, Kansas City, Mo. (Will supply "Protamone" for Subproject C)

10. Financial Support:

a. Proposed Budget 7-1-47 ..... 6-30-48 .....

Items	ALLOCATION OF FUNDS					
	Bankhead-Jones	Purnell	Adams	State	Other (1)	Total
1. Salaries						
P. I. Elliott				5,000		
D. W. Colvard				1,250		
Helen T. Ohmer				1,320		
2. Labor				100	~~1,000~~	800
3. Travel				570	300	
4. Equipment & Supplies				700	~~2,625~~	2600
5. All Other				490	500	
Total				9,430	~~4,425~~	4200

(1) Hope-Flannagan

b. Proposed Future Budgets:

Year	Salaries	Total Expenditures	Estimated Income
1947-48	7,570	13,855	
1948-49	8,770	20,000	
1949-50	8,770	20,000	

11. General Remarks:

Project No. RM2-A12

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date June 6, 1947 — F D Elliott

Title Assoc Prof J Dairy Husbandry

Date June 4, 1947 — P E Gwinnett

Title

Date

Title

2. Approval of Heads of Departments or Coöperating Agencies

Date June 7, 1947 — J H Hilton

Head Dept of Animal Industry

Date June 7, 1947 — A C Coward

Head, Dairy Husbandry Section

Date

Head,

3. Approval of Committee on Experiment Station Projects

Date

Chairman of Committee

4. Approval of Director

Date 6/30/47 — L D Baver

Director, North Carolina Agricultural Experiment Station

5. Approval of U. S. D. A.

Date AUG 7 1947 — E W Allen

Assistant

Chief, Office of Experiment Stations

1. Proposed Additional Sub-Project (E) to Master Project: "Studies of Factors Affecting the Reproductive Processes of Farm Animals".

Project No.: RM2-Ai2

2. Objectives: (a) To determine whether there are differences among various forage plants, especially alfalfa and grasses, fresh and cured, as sources of nutrients for bulls in reproductive service.

(b) To determine the effect of sustained general fever during calfhood on the subsequent fertility of dairy sires. McLaughlin -

(c) To determine whether there is a positive effect on fertility from the oral administration of synthetic thyroprotein ("Protamone") to dairy cows of low fertility.

(d) In general, to attempt to bring to light some of the factors which: (1) cause differences in fertility rates among various animals of a species, and (2) cause periodic variations in the fertility of individual animals.

(e) To attempt to develop laboratory methods by which reasonably accurate estimates of a bull's fertility can be made in lieu of actual fertility studies. Bleds ew's

3. Reasons for undertaking Investigations : It is well known that the maintenance of continuous optimum fertility in our domestic animals is a major problem which faces all practical husbandmen. Large amounts of potential income are lost each year to livestock men in North Carolina as elsewhere as a result of sterility or irregular breeding among the animals they own.

Titles of Subprojects A through D

Subproject A - A Comparison of Legumes and Grasses, Fresh and Cured, as Sources of Nutrients for Bulls in Breeding Service.

Subproject B - Effect of High Fever During Calfhood on the Subsequent Fertility of Bulls.

Subproject C - Effect of Feeding Synthetic Thyroprotein (Protamone) to Dairy Cows of Low Fertility.

Subproject D - The Development of a Laboratory Test for Semen Quality With a High Prediction Value as to the Fertility of Semen Samples.

"See Outline of Master Project for Details."

SUBPROJECT E.

1. Subtitle: Studies on the Effect of Environmental Temperature and Light on Reproductive Efficiency of Dairy Animals.

2. Objectives: (a) To determine to what extent light, ambient temperature and related factors (e.g. humidity) affect reproductive processes.

(b) To further determine the range in each of these factors within which optimum fertility can be maintained.

(c) From a practical point of view, to determine whether it would be advantageous to maintain valuable breeding animals (e.g. bulls in artificial breeding establishments) under controlled atmospheric conditions.

(d) To study the various breeds, strains or lines of dairy cattle in an effort to determine whether there are differences among them with respect to their response to these factors.

3. Reasons for undertaking investigations:

It has been established that there are seasonal variations in breeding efficiency under both natural and artificial breeding methods. It is also known that the reproductive processes of various members of the animal kingdom are stimulated (or retarded) by environmental factors such as light and/or temperature. The part that one or the other of these factors plays in the reproduction of the bovine species is practically unknown.

Once we know what factors have the greatest influence on reproductive processes, we can then act accordingly with respect to obtaining optimum fertility, as far as the following are concerned:

1. Controlling environmental conditions for particularly valuable breeding animals, and

2. Selecting for propagation lines, strains or breeds which are most nearly adapted to our conditions.

4. Previous work:

Light (Mercier and Salisbury, Jour. Dairy Sci. 30: 747, 1947) ,

Temperature and "Season" (Erb, Andrews and Hilton, Jour. Dairy Sci. 25: 815, 1942) appear to affect reproductive efficiency in the bovine. The extent of the effect of each of these factors has not been determined.

5. Outline of Procedure:

a. Temperature Studies

(1) Eight young bulls of 12 to 18 months of age will be divided as evenly as possible (randomized within breeds, etc.) into two groups. One group will be kept under ordinary herd conditions, the other in specially devised rooms where temperature can be controlled (experimental group).

(2) As a first step, the experimental group will be subjected to a temperature at wide variance from the mean temperature of the main barn (Hot in winter, cold in summer) over a period of 2 or 3 months.

(3) Records will be kept of heart rate, bodyweight, feed consumption, blood composition, etc., as well as of semen characteristics. Temperature records, and other seemingly pertinent environmental data, will also be recorded.

(4) Following the preliminary data obtained with respect to the kind of effect produced by temperature, studies will be continued in an effort to locate breaking or critical points, i.e., to determine over what range of environmental temperature the reproductive processes of the bull operate at their optimum efficiency.

b. Light Studies

Similar investigations, using appropriate methods and equipment, will then be carried on with light and/or humidity as the variable.

c. Studies of the response of various breeds, strains, and lines will be carried on after methods and measuring devices have been developed.

6. Probable Duration of Project: 10 - 15 years

7. Date of Initiation: July 1, 1949

8. Personnel:

Name	Department	Relation to Project
F. I. Elliott	Animal Industry (Dairy)	Leader
C. D. Grinnells	Animal Industry (Dairy)	Cooperator
H. A. Stewart	Animal Industry (An. Hus.)	Cooperator
D. W. Colvard	Animal Industry	Adviser

9. Cooperation:

a. Interdepartmental - Institute of Statistics

b. Other Agencies - U. S. Weather Bureau

R. A. McGinty

At last meeting of Com of 9
South was allotted ⅙ of Regional funds
9B3 $20,000 to go to Dairy Breeding project
Southern Director will allocate
by states

$5000 to Texas, Fa, S.C. & Tenn
(Recommended by McGinty & LaMaster)

Passed 6000 SC (1000 trimmed)
5000 Texas
3000 Fa
3000 Tenn
3000 Fla.
20,000

Check on heat tolerance test developed for Gulf-
Prepare project on Purebred Jerseys - Line project-
Copy to McGinty for 10 Pr
to Dr Woods & to Committee of Tech Committee -
then Foreman - with request for funds -

Next Meeting

FINAL REPORT, COMPLETED OR REVISED PROJECTS

North Carolina Agricultural Experiment Station

1. Project title, number, and fund: RESEARCH & MARKETING An. Ind. NE2-Ai2, STUDIES OF FACTORS AFFECTING THE REPRODUCTIVE PROCESSES OF FARM ANIMALS. B. THE EFFECT OF CALFHOOD FEVER UPON SUBSEQUENT FERTILITY IN DAIRY BULLS.

2. Departments and cooperating agencies:
Department of Animal Industry

3. Major personnel: D. W. Colvard, R. E. Waugh, A. L. McLaughlin, and T. C. Blalock

4. Date begun: December 1948 Date revised/completed: March 1950
If discontinued without completion state reasons:

5. Estimated total cost by funds (salaries and maintenance): $1800

6. The problem (briefly restate its nature, importance, and economic significance):
The experiment was designed to measure the effect of calfhood fever on subsequent fertility in dairy bulls. During the course of the study two testis biopsies were performed and since reports have been published showing a harmful effect being produced by the biopsy itself, the bulls were continued in an effort to determine if this had been the case in these bulls.

It is important to know whether or not young bulls having cases of high fever due to calfhood diseases may be expected to perform as normal breeders. This will aid the breeder in deciding whether to keep promising bull calves after having a high fever or whether he should discard them. The biopsy technique is a valuable tool for the research man but to use it he must be sure the answers he obtains are not a result of effects produced by the biopsy.

7. Major results and conclusions:
No differences could be shown between the semen quality of bulls given calfhood fevers and those carried as controls with no fever treatment. Therefore, it seems that normal breeding efficiency can be expected from bull calves that have experienced high fevers during calfhood.

The effects from the biopsy were variable. Three of the six bulls definitely seemed to be adversely effected. The remaining three showed a slight decrease in some semen characteristics but could still be considered as normal bulls. The testicle of one of the bulls atrophied following the first biopsy and after the second biopsy spermatogenesis practically ceased.

From these results, it would seem that in using the biopsy technique, the investigator must be aware of the effects that may be produced and interpret his results accordingly.

(over)

* Two copies to be sent to the Office of Experiment Stations.

8. Practical applications and public benefits achieved or in prospect:

From the results obtained on the effect of calfhood fever on subsequent fertility, the breeder need not discard promising bulls that have had a high fever.

These results indicate that the biopsy technique can be used in experimental work and will add considerable information in many experiments.

9. Publications:

The Effects of Artificially Induced Calfhood Fever Upon the Subsequent Fertility of Dairy Bulls. A. L. McLaughlin, F. I. Elliott, and D. W. Colvard.

Director

OES-Form-17 (Rev.)
(January 10, 1951)

North Carolina Agricultural Experiment Station

FINAL REPORT, FEDERAL-GRANT PROJECTS
(Send 3 copies to Office of Experiment Stations at time of closing)

1. PROJECT (Fund, number and title): R & H 2-M12-D, Studies of Factors Affecting Reproductive Processes of Farm Animals.--D, The Development of a Laboratory Test for Semen Quality with a High Prediction Value as to the Fertility of Semen Samples.

2. STATION DEPARTMENTS AND COOPERATING AGENCIES (e. g., USDA, TVA, etc.): Animal Industry.

3. MAJOR PERSONNEL: F. I. Elliott, R. K. Waugh, R. H. Ruffner, and T. C. Blalock

4. DATE BEGUN: Never active. DATE COMPLETED:
(If discontinued without completion state reasons): After preliminary work, the major personnel involved left this station and the project was not continued.

5. ESTIMATED TOTAL COST BY FUNDS (Federal-grant and others):
None.

6. THE PROBLEM (Briefly restate its nature, importance, and economic significance): To compare semen quality tests now in use from the point of view of their value for routine use. To attempt to develop a test which will be simple, quick and which will give results with a high correlation to actual fertility. At present no lab test is available which will give a reliable indication as to the fertility of a semen sample when it is used to breed cows. The availability of a reliable test would be a great boon, both to artificial breeding associations, and to research.

7. ABSTRACT MAJOR RESULTS AND CONCLUSIONS: None.

(Over)

8. PUBLIC BENEFITS (Present or potential, realized by public, stated in dollars, bushels or other values): None.

9. CITATION OF PUBLICATIONS (Issued and/or in manuscript form): None.

10. Prepared by Robert E. Cassady Approved _____ (Director).

(Sign original only)

Date April 17, 1951 Date _____

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 19 50
(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): RESEARCH & MARKETING Am.Ind. RM2-Ai2-D, STUDIES OF FACTORS AFFECTING THE REPRODUCTIVE PROCESSES OF FARM ANIMALS.--D, THE DEVELOPMENT OF A LABORATORY TEST FOR SEMEN QUALITY WITH A HIGH PREDICTION VALUE AS TO THE FERTILITY OF SEMEN SAMPLES.

2. DEPARTMENTS AND COOPERATING AGENCIES: Department of Animal Industry

3. PERSONNEL: F. I. Elliott, R. K. Waugh, R. H. Ruffner, and T. C. Blalock

4. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked):

Analysis of correlation between the test developed and other semen quality tests run, showed no positive relationship. Due to a delay in obtaining a glass electrode necessary for further work there has been no progress on this project.

5. BENEFITS realized by farmers or the public through application of findings, stated in dollars, bushels, or other values, where possible:

No benefits realized as yet, but the availability of a reliable and convenient semen quality test would be worth inestimable amounts to dairymen and artificial breeding organizations throughout the entire United States.

6. WORK PLANNED FOR NEXT YEAR:

In vivo pH determinations will be made on the cervix and uterus of the cow, which may result in changes in the pH level of the medium used. The test will be run on semen samples being used to breed a large number of cows in an attempt to correlate the results with actual fertility results.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. APPROVED: R K Waugh
Project Leader. Director.

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1950
(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): RESEARCH & MARKETING An.Ind. RM2-A12, STUDIES OF FACTORS AFFECTING THE REPRODUCTIVE PROCESSES OF FARM ANIMALS.
B. THE EFFECT OF CALFHOOD FEVER UPON SUBSEQUENT FERTILITY IN DAIRY BULLS.

2. DEPARTMENTS AND COOPERATING AGENCIES:
Department of Animal Industry

3. PERSONNEL: D. W. Colvard, H. K. Waugh, A. L. McLaughlin, and T. C. Blalock

4. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked): Eight bulls were used in this experiment, four being given fever artificially by injections of a mixture of sulfur in olive oil, and four carried as controls with no fever treatment. The average daily temperature of the treated bulls was increased 2° to 3° above normal for about three weeks.

Twelve semen samples were collected from each bull and evaluated by certain tests for semen quality. The two groups were compared and no statistical difference could be noted between the two groups.

Histological examination of stained slides of tissue taken from the testicle by means of biopsy both before the treatment and following the collection of the last sample of semen revealed no differences.

In addition to the four pairs of experimental animals, one additional animal was used. This bull had a natural fever due to a respiratory ailment when he was a young calf. He was not included in the average of the fever group but when compared by inspection with the average of the control group no difference could be noted.

These results give no indication that fever during calfhood has any effect on later semen production.

Recently results have been published to show that two successive biopsies on mature bulls resulted in a marked decrease in semen quality, especially following the second biopsy. (Over)

5. BENEFITS realized by farmers or the public through application of findings, stated in dollars, bushels, or other values, where possible:

Undetermined

Voided

6. WORK PLANNED FOR NEXT YEAR:

None

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

The Effects of Artificially Induced Calfhood Fever Upon the Subsequent Fertility of Dairy Bulls. - A. L. McLaughlin, F. I. Elliott, and D. W. Colvard - Presented at a meeting of the Southern Section of the Dairy Science Association in 1950.

8. APPROVED: _____ _____
Project Leader. Director.

No. 4 continued

In this experiment, following the first biopsy, the testicle of one of the bulls atrophied. No swelling or inflamation was observed but the biopsied testicle did not continue to grow. A later sample taken from this same testicle showed it to be mainly connective tissue. Semen quality in this bull did not seem to be effected. Following the second biopsy however, a very marked decrease in semen quality occurred. In fact, samples were collected in which no sperm could be found and motility was nearly always absent.

Twelve samples were collected from all bulls following the second biopsy. The data is now being analyzed and with the exception of the one bull whose testicle atrophied, there seems to be no great difference in semen quality between samples collected following the first biopsy and those taken after the second biopsy. These results would indicate that the biopsy technique may be used in experimental work with young bulls but investigators should be aware that this technique may produce abnormal results.

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1949.
(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): RAM RM2-A12-D, STUDIES OF FACTORS AFFECTING THE REPRODUCTIVE PROCESSES OF FARM ANIMALS.---D, THE DEVELOPMENT OF A LABORATORY TEST FOR SEMEN QUALITY WITH A HIGH PREDICTION VALUE AS TO THE FERTILITY OF SEMEN SAMPLES

2. DEPARTMENTS AND COOPERATING AGENCIES: An. Ind.

3. PERSONNEL: F. I. Elliott, C. D. Grinnells, R. K. Waugh, R. H. Ruffner, D. W. Colvard, and T. C. Blalock.

4. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked):

Fifteen semen samples were collected from bulls at the College Farm. The group included bulls of both high and low fertility. Each semen sample was split into several parts. Comparisons were made on portions of the same semen ejaculate, between mediums (Na_2HPO_4 and KH_2PO_4) buffered at pH 8.0 and pH 8.4. Temperatures ranging from 37.5° C. to 49.0° C. were tried. The critical temperature appeared to be 48.0° C., with higher temperatures resulting in early death of the spermatozoa.

Motility estimates were made at 5-minute intervals for the first half-hour, then at 15-minute intervals, and the time that progressive motility ceased and the time of death of all sperm being noted.

Attempts were made to correlate the results of the test with results obtained, using other tests already developed. The latter included: the methylene blue reduction time, length of survival of regularly diluted semen in water bath at 46.5° C. (G. W. Salisbury et al.), at 37.5° C. (T. Ludwick), and at 5° C. Original motility and concentration estimates were made on each fresh sample.

5. BENEFITS realized by farmers or the public through application of findings, stated in dollars, bushels, or other values, where possible:

No benefits realized as yet, but the availability of a reliable and convenient semen quality test would be worth inestimable amounts to dairymen and artificial breeding organizations throughout the entire United States.

6. WORK PLANNED FOR NEXT YEAR:

In vivo pH determinations will be made on the cervix and uterus of the cow, which may result in changes in the pH level of the medium used. The test will be run on semen samples being used to breed a large number of cows in an attempt to correlate the results with actual fertility results.

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. APPROVED: F I Elliott
Project Leader. Director.

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION

ANNUAL PROGRESS REPORT, FEDERAL-GRANT PROJECTS, 1949.
(Three copies to be given to the OES examiner)

1. PROJECT: (Fund, number, and title): RAM RM-2-A12, STUDIES OF FACTORS AFFECTING THE REPRODUCTIVE PROCESSES OF FARM ANIMALS

2. DEPARTMENTS AND COOPERATING AGENCIES: An. Ind.

3. PERSONNEL: F. L. Elliott, C. B. Grinnells, R. E. Waugh, R. H. Ruffner, D. W. Colvard, A. L. McLaughlin, and T. C. Blalock

4. NATURE OF WORK AND PRINCIPAL RESULTS OF THE YEAR (Confidential information should be so marked):

Subproject A - Work on this phase still awaits the development of facilities.

Subproject B - Testicular biopsy specimens were taken from the left testis of each of the nine bulls on May 17, 1948, and the tissue prepared for histological study. Fever treatment was begun on May 27, 1948. Dosages of 25 to 35 mg. S/Kg body weight were used. Double this amount, given in two injections, did not produce greater fever. The maximum fever temperature obtained was 105.2° F., and the daily temperatures of the treated animals averaged 0.6° to 1.5° F. higher than those of the control calves. Treatments were stopped on July 14, 1948.

Subproject C.- Pending the availability of facilities for studying the absolute effects of various levels of thyroid activity on reproductive processes, this phase of the work has been suspended.

5. BENEFITS realized by farmers or the public through application of findings, stated in dollars, bushels, or other values, where possible:

It is becoming more generally recognized that fertility, or lack of it, is one of the primary factors affecting the efficiency of our animal husbandry in general. Large-scale regional projects to study this problem have been established with the cooperation of the Department of Agriculture, and the Dairy Cattle Breed Associations are now developing a Research Foundation which (Over)

6. WORK PLANNED FOR NEXT YEAR:

Subproject A - Barn space will be available shortly, and the work will be initiated during this coming year.

Subproject B - Semen collections have been delayed pending the completion of more adequate facilities. The bulls were moved into their new quarters this past week (March 5). Indications are that the biopsy specimens may confuse the picture as data presented subsequent to the biopsy by J. F. (Over)

7. PUBLICATIONS ISSUED OR MANUSCRIPTS PREPARED DURING THE YEAR:

None

8. APPROVED: F. L. Elliott
Project Leader.

Director.

5. (Continued)

will sponsor work in this field. The particular work being discussed herein has not yet progressed far enough to have made a definite contribution, however.

6. (Continued)

Sykes et al. of the Bureau of Dairy Industry, U. S. Department of Agriculture, indicates that testicular biopsy may result in atrophy and a decrease in ~~testicular~~ spermy concentration. It is worth noting, however, that in only one case has testicular atrophy occurred.

Semen collections, followed by second biopsies, will be carried out during this spring, as previously planned.

Subproject C - The work planned for last year will be carried out if facilities and personnel are available.

NORTH CAROLINA AGRICULTURAL EXPERIMENT STATION
PROJECT OUTLINE

Addition to

Project No. RM2-Ai2 .....
Date
Submitted .....
Approved .....
Revised .....

1. Title :

Studies of Factors Affecting the Reproductive Processes of Farm Animals.

2. Objective(s) : (a) . . . . .)
(b) . . . . .)
(c) . . . . .) (See Master Project Outline)
(d) . . . . .)
(e) To attempt to develop laboratory methods by which reasonably accurate estimates of a bull's fertility can be made, in lieu of actual fertility studies.

3. Reasons for undertaking Investigations*

(See Master Project Outline, also detailed outline of this Subproject which follows on page 2.)

*Including economic justification

SUBPROJECT D

1. Subtitle: The Development of a Laboratory Test for Semen Quality With a High Prediction Value as to the Fertility of Semen Samples.

2. Objectives: (a) To compare semen quality tests now in use, from the point of view of their value for routine use.

(b) To attempt to develop a test which will be simple, quick, and which will give results with a high correlation to actual fertility.

3. Reasons for undertaking investigations:

At present no laboratory test is available which will give even a relatively reliable indication as to the fertility of a semen sample when it is used to breed cows. Consequently, the only way to obtain an indication as to the fertility of a bull is to breed cows with his semen, and then observe the number or percentage which conceive.

The availability of a reliable test would be a great boon, both to artificial breeding associations, and to research units. Infertile bulls could be removed from studs much earlier, resulting in decreased loss to dairymen because of delayed breeding. Experimental studies could be carried on, even without large numbers of cows being available for testing semen samples.

The other phases of this project will be greatly facilitated if such a test becomes an actuality. Hence this work is really fundamental to the other work in progress.

4. Previous work:

Various so-called semen quality tests are now available. They include motility estimates (microscope), concentration of spermatozoa, volume of ejaculate, the methylene blue reduction test, the cold-shock test, livability at 3° C., 37.5° C., and 45 to 50° C., and measurements of glycolysis and respiration. The correlation of results on these tests and actual fertility is woefully small, or not significant.

5. Outline of procedure:

(a) Acquaintance with the techniques of the three or four best tests now used will be developed under laboratory conditions.

(b) Preliminary to development of the semen evaluation test as outlined below, the pH of the cow's reproductive tract, especially the uterus during estrus, will be studied. If possible, a technique for making in vivo pH determinations will be devised.

(c) An attempt will be made to develop a test based on incubation at a high temperature (47° C. or higher), but in which the diluent will be so devised

as to simulate the fluids of the reproductive tract of the cow in estrus, especially with respect to hydrogen ion concentration.

(d) Tests will be run on semen which is being shipped from a large stud (Southeastern Artificial Breeding Association, Asheville, N. C.), and correlation studies made between results on the tests and actual fertility or non-return reports.

6. Probable Duration of Project: 2 years

7. Date of Initiation: July 1, 1948

8. Personnel: No changes

Name	Department	Relation to Project
F. I. Elliott	Animal Industry (Dairy)	Leader
C. D. Grinnells	Animal Industry (Dairy)	Co-leader
R. K. Waugh	Animal Industry (Dairy)	Cooperator
R. H. Ruffner	Animal Industry (Dairy)	Adviser
D. W. Colvard	Animal Industry	Adviser

9. Coöperation:

a. Interdepartmental

b. Other Agencies - Southeastern Artificial Breeding Association, Asheville, N.C.

10. Financial Support: Additional funds under RM2-Ai2.

a. Proposed Budget 7-1-48 to 6-30-49

Items	ALLOCATION OF FUNDS					
	Bankhead-Jones	Purnell	Adams	State	Other (1)	Total
1. Salaries					1,800	
2. Labor					450	
3. Travel					400	
4. Equipment & Supplies					700	
5. All Other					350	
Total					$ 3,700	

(1) Research & Marketing

b. Proposed Future Budgets: In addition to present budget.

Year	Salaries	Total Expenditures	Estimated Income
1949-1950	$2,000	$ 4,000	

11. General Remarks:

No other changes in setup of the project are being made at this time.

Project No. RM2Ai2D.

SIGNATURES OF APPROVAL

1. Approval of Project Leaders

Date July 22, 1948 — J. D. Elliott (NW.S.)
Title Leader

Date July 22, 1948 — C. D. Grinnells
Title Co-leader

Date .....
Title .....

2. Approval of Heads of Departments or Coöperating Agencies

Date July 22, 1948 — D. H. Colvard
Head, Dept. of Animal Industry

Date .....
Head, ...

Date .....
Head, ...

3. Approval of Committee on Experiment Station Projects

Date .....
Chairman of Committee

4. Approval of Director

Date July 23, 1948 — R. W. Cummings
Assoc. Director, North Carolina Agricultural Experiment Station

5. Approval of U. S. D. A.

Date Aug 6, 1948 — W. C. Krauland
Acting Chief, Office of Experiment Stations