OKLAHOMA
AGRICULTURAL AND MECHANICAL
COLLEGE

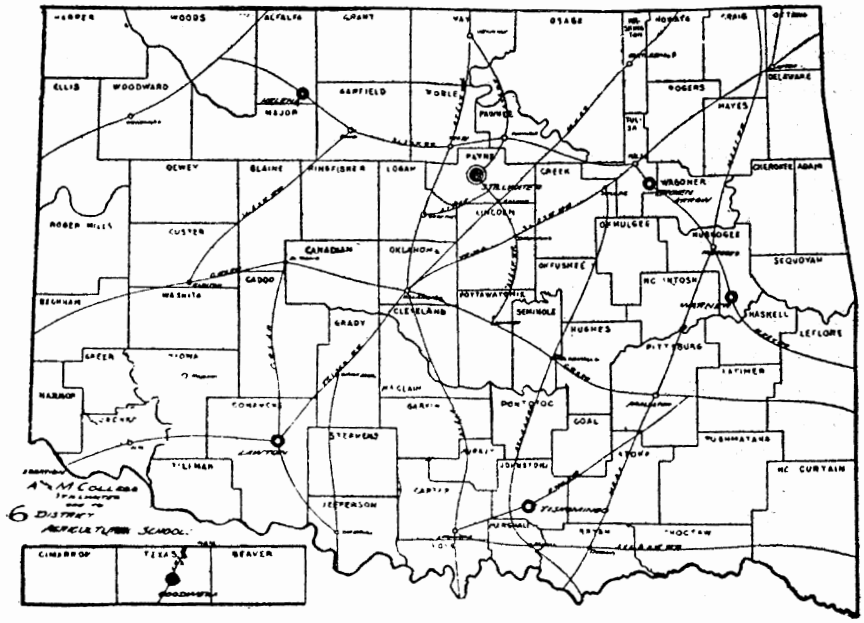
AGRICULTURAL EXPERIMENT STATION

BULLETIN NO. 96 DECEMBER, 1911

THE VITALITY OF REPRODUCTIVE CELLS

BY
LOWERY L. LEWIS

VETERINARY



LOCATION OF THE A. & M. COLLEGE AND THE SIX SCHOOLS OF AGRICULTURE

STILLWATER, OKLAHOMA

STATE BOARD OF AGRICULTURE

(Ex-Officio Board of Regents)

HON. G. T. BRYAN, *President*.....Perry
HON. DAN DIEHL, *Vice President*.....Gotebo
HON. R. F. WILSON, *Treasurer*.....Valliant
HON. J. C. ELLIOTT.....Pauls Valley
HON. EWERS WHITE.....McLoud
HON. L. G. GRIFFIN.....Marlow
HON. J. W. L. CORLEY.....Howe
HON. A. C. COBB.....Wagoner
HON. O. A. BREWER.....Helena
HON. GEORGE H. HINDS.....Westville
HON. FRANK L. HAYMES.....Broken Arrow

J. H. CONNELL, M. S., *President of the College*

STATION STAFF

JAMES A. WILSON, B. Agr.....*Director*
L. L. LEWIS, M. S., D. V. M.....*Veterinarian and Bacteriologist*
W. A. LINKLATER, B. S. A.....*Animal Husbandman*
ROY C. POTTS, B. S.....*Dairyman*
C. E. SANBORN, M. A.....*Entomologist*
CHAS. K. FRANCIS, Ph. D.....*Chemist*
N. O. BOOTH, B. Agr.....*Horticulturist*
O. O. CHURCHILL, B. S.....*Agronomist*
A. H. WRIGHT, B. S.....*Assistant Agronomist*
R. O. BAIRD, B. S.....*Assistant Chemist*
D. C. MOORING, M. S.....*Assistant Horticulturist*
C. I. BRAY, M. S.....*Assistant Animal Husbandman*
A. A. JONES, M. S. A.....*Assistant Chemist*
T. E. MCELROY, B. S.....*Assistant Entomologist*
B. J. CLAWSON, M. S.....*Assistant Bacteriologist*
J. L MCKEOWN.....*Financial Secretary*
W. W. EVANS.....*Farm Superintendent*
LULA TOURTELLOTTE.....*Clerk*

VISITORS ARE CORDIALLY WELCOMED AT ALL TIMES

The publications of the Station are sent free to residents of Oklahoma on request. All communications should be addressed, not to individuals or departments, but to the

EXPERIMENT STATION, Stillwater, Oklahoma.

THE VITALITY OF REPRODUCTIVE CELLS

BY L. L. LEWIS
Professor of Veterinary Science

INTRODUCTION

The experiments recorded in this bulletin are the outgrowth of work that was begun in 1904. The work was begun with the idea of demonstrating the possibilities of artificial insemination in the breeding of animals, especially in horse breeding. In connection with this study the work has developed along related lines, especially the study of the vitality of the sperm cell under varying experimental conditions as well as the study of the vitality of the cell under normal conditions. The handling of the semen by means of instruments in artificial insemination and thus exposing it to artificial conditions has led to the careful study of the vitality of the sperm cell under a great variety of laboratory conditions.

Two brief publications have already been issued dealing with the subject in a general way. Circular No. 5 was issued in March, 1905, and Bulletin No. 93, which was recently issued, dealt briefly with work reported more fully in this bulletin.

There are many important questions bearing on animal breeding that should be solved in order to make our work in this line more certain and satisfactory. In 1905 the writer, in cooperation with Mr. W. L. English, then assistant in animal husbandry, conducted some preliminary laboratory studies of the vitality of the semen from the horse as affected by temperature. Some of the results obtained suggested that the method of artificial impregnation, or, more correctly, artificial insemination, could be practiced extensively in horse breeding. These studies have been continued, not only along this line, but also along other important lines, in connection with the subject of animal breeding. A number of problems have been touched upon that have not been sufficiently tested to justify publication at this time, while a number have been studied sufficiently to justify a preliminary report. This report of the work is made more with the idea of stimulating interest and investigation by others along this line of work than to announce definite conclusions in regard to any certain phase of the work that has been undertaken. It is hoped to

continue these experiments until many of the important problems relating to animal breeding have been solved. Definite knowledge is lacking regarding many of these problems. It is known that certain special cells from the reproductive glands of the male and from the female are necessary for propagating the species, but as to the length of time these cells will live in the body of the animal or the exact influence or conditions tending to either prolong or shorten their life there is very little information. Again, it is usually assumed that the ovum or egg cell of the female is liberated from the ovary at the first signs of heat, or oestrum, but definite information in regard to this matter is lacking in most cases. Whether the animal should be bred during the first or last period of heat may, after all, be of much greater importance than it is supposed to be by most breeders. Certain physiological laws are involved in the process of fertilization and any undue external influence or abnormal conditions of the organs of either sex may retard or even prevent fertilization. From a practical point of view it is not so much the process of fertilization itself that we need to understand, but the influences and conditions favoring this process; to know when the egg cell is liberated from the ovary and is therefore capable of being fertilized; and, to know as definitely as possible the influences of feed, exercise, and care upon the vigor of the reproductive cells and upon the process of fertilization. Many of these problems need to be studied and may be studied in an experimental way.

The condition of the reproductive glands is an important feature of investigations of this character, and no doubt much may be learned by histological studies of these glands, especially when they are taken from individuals of known breeding qualities.

The semen from the horse and hog was used in the major portion of this work. The relatively large size of the sperm cell and its active condition when first obtained from these animals enables one readily to determine the effects of any experimental conditions that may be imposed, so far as these may affect vitality. No exact method has been found to determine the per cent of moving cells in any given preparation, but in all cases the per cent given is an estimate made by a careful inspection of the field of any given preparation.

VITALITY OF THE SPERM CELLS WITHIN THE BODY OF THE FEMALE

It is often asserted by the breeder and others who are familiar with many of the various problems connected with animal breeding, that the sperm cells will live for days or even weeks in the organs of the female. It is very important to know the length of time the sperm cells will live under natural conditions, as this information, taken in connection with a knowledge of the time at which the egg cells of ovules escape from the ovary, makes our information in connection with certain phases of animal breeding definite instead of a matter of guesswork. The vitality of the sperm cell outside of the body, or under laboratory conditions, no matter how favorable these conditions have been, is a question of hours and not one of days or weeks. While there is of course an incalculable difference so far as conditions are concerned, in the organs of the female and in the conditions imposed by laboratory experiments, yet as the result of these experiments, both laboratory and actual breeding work, the sperm cells have been found to be short-lived. In addition to this, these cells are extremely sensitive to any unfavorable influences such as the presence of other fluids, as urine or water, or to the presence of chemicals in very small amounts. In general it may be said that variations of temperature exert a less harmful effect upon the vitality of the sperm cells than any other condition imposed by the experiments.

In order to determine the length of time the cells will live in the body of the female a number of sows were killed at varying lengths of time after they were bred, and microscopic examinations were made of the organs for live sperm cells; the ovaries were also examined in order to learn as nearly as possible the time at which the ovum was liberated from the ovary. The plans and details of these experiments will be considered more fully under another division. In the experiments that were carried out to determine the question of vitality of the sperm cells it was found that there was a difference of only a few hours in the length of time these cells will live under laboratory conditions and the length of time they will live in the body of the animal when introduced either by service or by artificial means, such as gelatine capsules, parchment bags, etc. However, there is a great variation in the vitality of the sperm cells from different individuals, as well as some variation in the vitality of the sperm cells from the same individual at different times. The experiments under this head are logically divided into two groups. In

the first group are placed those experiments conducted during the years 1908 and 1909, and in the second group those conducted in 1910 and 1911. This division is necessary so far as the work relates to hogs on account of the individual difference in the males used in the experiment.

Vitality Tests:—In order to test the vitality of the sperm cells in the organs of the mare, breeding capsules, celloidin sacks, rubber bags and parchment bags were used to introduce the semen into the vagina and womb. On February 2, 1908, semen was collected from a stallion, and fifteen cubic centimeters (one-half an ounce) was placed in each of two celloidin capsules after which they were sealed. Two mares were used and each had a capsule of the semen placed in the womb. The semen was collected at noon and was divided and used in the experiment as soon as possible, probably within less than ten minutes. At 5 p. m. an examination was made to determine if any of the cells were alive. Neither of the capsules placed in the womb was found in place, having been forced into the vagina. A careful examination was made of the capsules and no live sperm cells were found in either of them. The colloidin capsules were very thin and would allow the passage of body fluids into the semen. On May 8 another lot of semen was secured from the same stallion and twenty cubic centimeters were placed in a bag made of rubber and inserted into the vagina. After a lapse of five and one-half hours the material was examined and no live cells could be found. A portion of this lot of semen was taken to the laboratory and kept at 25° C. At 4 p. m. the lot kept in the laboratory showed 45 per cent alive, while that kept in the body of the mare for the same length of time showed no live cells. At the time the experiment was made it was thought that the results merely showed the effect of the different temperatures, as the rubber prevented any of the body fluids mixing with the semen. Further tests have been made along this line by using thin rubber obtained from breeding bags, and it has been found that semen left in contact with the rubber for any length of time will lose its vitality quicker than samples of the same lot that are placed in glass, parchment, or gelatin capsules.

A knowledge of the vitality of sperm cells is very important in breeding work, especially when the use of artificial means by which the semen may be introduced into the organs of the female is to be considered. These experiments have not yet determined whether the loss of the ability to fertilize is coincident with the apparent loss of vitality as determined by microscopic examination, or whether the ability to fertilize is lost for some time before movement finally ceases. When it is considered that under a great variety of laboratory conditions tests of the vitality of the cells when placed in the organs of the female in bags of parchment, celloidin, etc., and the examination of the organs from a number of sows killed at varying lengths of

time after breeding, all confirm the statement that the sperm cells of some of the domestic animals are short-lived under either normal or abnormal conditions, it is evident that a definite knowledge of the vitality of the male cells and of the conditions affecting this vitality will be of immense advantage in animal breeding. The idea is quite common among breeders that the sperm cells will live for several days in the organs of the female. This belief is so firmly fixed in the minds of some breeders that they will breed mares out of season expecting that the sperm cells will be present when the period of heat arrives and that the egg or ovum will be fertilized from a service several days previous. If any results follow breeding of this kind they may be explained on one of two suppositions: One would be that ovulation was not coincident with the ordinary signs of heat, which undoubtedly may be true occasionally, and especially so in the mare. The other supposition would be that the egg cell or ovum produced at the last period of heat was retained in the womb and was still capable of being fertilized. If it is assumed that the escape of the egg from the ovary in the mare or sow is coincident with the ordinary signs of heat, then breeding between periods of heat will be without results unless the ovum is retained for a number of days after the period of heat has passed, as the sperm cells do not appear to live for a sufficient length of time to enable them to fertilize an ovum produced at a subsequent period of heat, unless the liberation of the egg should follow within a short time after breeding. The statement is also made that the sperm cells will live for days in the organs of the male after death or after removal of the organs by castration.

In order to get some idea of the length of time the cells would live in the gland the following observations were made: Three young boars were castrated and examinations made of the testicles. One of them was a poor breeder used in the breeding experiments with sows twenty-one to twenty-five where the sperm cells could not be found on postmorten examinations made a few hours after they were bred. The other hogs were vigorous young males weighing about 250 pounds each. After castrating the hogs the testicles were taken to the laboratory and kept at room temperature—22° C. Material for examination was taken from the body of the gland, from the epididymus, and from the spermatic cord. In the testes from normal males the vitality of the sperm cells decreased in about the same manner as they would if kept in a glass vessel and under the same conditions of temperature. After three hours there was approximately 60 to 70 per cent alive. At this time the observations were discontinued until the next morning, or eighteen hours after the glands were removed. A very careful examination at this time failed to show any of the cells alive. In the glands secured from the male that was a poor breeder practically all of the cells were dead after three hours. There is little doubt but that practically the same

results would be obtained by an examination of the testes of the stallion or bull.

A thorough knowledge of the time that ovulation takes place as compared with the first signs of heat, combined with a knowledge of the vitality of the sperm and of the conditions in the body of the female affecting vitality, are matters that need careful investigation in all cases. Breeding should be placed on a scientific basis and should keep pace with other lines of work. Scientific methods are practiced and careful studies made in practically all lines of work, but at present very little study has been given to this most important matter from the exact or scientific side. The problem has been treated very much as if there was nothing to learn, or else nothing to be gained in a practical way through scientific research. This does not seem to be a reasonable view to take of any question, much less one where so many failures result from some cause, and in most cases from causes that in all probability might be remedied. There are many animals that are considered barren that would have a great value if they could be bred, and it is only by a study of the various problems connected with animal breeding that we can hope to come to any conclusion as to the reason for many of the things that are now regarded as beyond our reach and therefore not to be remedied or interfered with in any manner.

Experiments on Hogs to Determine the Length of Time After Coming in Heat Before the Graffian Follicles Rupture and to Determine the Length of Time the Sperm Cells Will Live in the Reproductive Organs After Breeding. It was thought advisable in connection with the preliminary experiments to determine as nearly as possible the length of time after coming in heat before it was possible for the ovum or egg cell to become fertilized. The period of heat is usually associated with certain physiological conditions or changes in the ovary, and it is during this period of sexual excitement that the ovum escapes from the ovary into the oviduct and finally enters the uterine horn where, if it is fertilized, it develops into a new organism.

Hogs were selected for this work principally on account of the ease with which one may determine when the period of heat begins. Twenty-five sows were used in the experiment. Some of the sows had farrowed litters of pigs, some were non-breeders, and a considerable number were young hogs that had never been bred. The sows were kept in an open shed and a male was penned in one corner of the lot and was turned in among the sows once or twice daily. In those cases where the period of heat began during the day a very correct estimate could be made as to the time of coming in heat, but in those cases that were not in heat late in the afternoon, but were in heat the next morning, only an approximate estimate could be made of the exact time of coming in heat. In addition to learning the length of time after heat began before ovulation occurred the

sows were bred and killed at varying lengths of time after breeding in order to determine the length of time the sperm cells retained their vitality. By making microscopic examinations of scrapings from the uterus and uterine horns some data was gathered as to the length of time the sperm cells live in the organs of the sow under normal conditions.

The chances for error in observations of this character cannot be entirely eliminated. For example, it is impossible to examine scrapings from all portions of the body of the uterus or of the cornu within any reasonable time. A number of preparations were always made from various parts of the organs and experience in the work soon showed that we might easily expect to find sperm cells at the point of union of the oviduct and uterine horn. Material from this point was usually examined first, after which the uterine horns and body of the uterus were cut at various places and scrapings secured for examination. It will be seen that the possibilities of overlooking occasional live cells are very great. However, is seems that with the care that was taken in making the examinations and the average results obtained from a considerable number of cases that these experiments represent a fairly accurate and average estimate of the length of time that the sperm cells will live in the organs of the sow. There is no difficulty in determining whether the Graffian follicles are ruptured or not, consequently the time given in the table at the end of this topic as the time elapsing from the time the period of heat began until the follicles were ruptured may be considered as fairly representative for this group of animals. The time may vary in other groups of animals, as for example in sheep, cattle or horses.

One very noticeable feature of the examinations was the scarcity of sperm cells in the uterus within a short time after breeding. If the examinations were made within one or two hours after breeding an abundance of sperm cells were found and the per cent of live cells was accordingly high. As the length of time after breeding increases, the number of sperm cells found, as well as the number alive, decreases very rapidly until within a comparatively few hours after breeding not only will there be no live sperm cells found, but all of the sperm cells have disappeared. A microscopic examination of the scrapings from the body of the uterus or from the cornu will show a great number of leucocytes. Phagocytic action of the white cells upon the sperm cells was not noted, but it is possible that these blood cells do take an important part in the disappearance of the semen cells.

Post Mortem Notes, 1908-1909

The following notes are of a general nature and refer particularly to the conditions found on post mortem examinations made of the sows that were used in this experiment. In each case the date will

show the time of coming in heat, the time of breeding and the time the sow was killed. In cases where the time of coming in heat is given as some hour during the night it is of course the estimated time of the beginning of heat, but in the remainder of the cases the time may be taken as fairly accurate, as the hogs were kept under very close observation.

Case No. 1. Young sow, came in heat at 8 a. m. May 29, bred at 5:30 p. m. on same date and killed at 9:30 a. m. May 30. The sow was killed 25.5 hours after coming in heat and 16 hours after being bred. An examination of the ovaries showed three well developed follicles in the right ovary and four in the left ovary. None of the follicles were ruptured. Microscopic examination showed an occasional live sperm in the oviduct and a considerable number of dead sperm cells at the point where the oviduct enters the horn of the uterus. Examination showed no sperm cells in the lower portion of the uterine horns or in the vagina.

Case No. 2. Young sow, came in heat at noon on July 1, and was killed at 2 p. m. on July 3, or fifty hours after coming in heat. This sow was not bred. The right ovary contained three follicles and the left ovary five follicles. The follicles were all ruptured, showing that the eggs or ovules had already escaped into the oviduct.

Case No. 3. This was a young sow that came in heat at 3 p. m. on July 9, was bred at 3 p. m. on July 10, and killed at 1:30 p. m. July 11. She was killed 46.5 hours after coming in heat and 22.5 hours after being bred. The right ovary showed six follicles and the left ovary one follicle, none of them being ruptured. The surface of the follicles showed congested spots, indicating that they would have ruptured within a few hours. An examination for sperm cells showed a considerable number of dead cells at the cervix of the uterus, a few dead cells were found at the points along the uterine horn, and slowly moving cells were found where the oviducts enter the uterine horns.

Case No. 4. This was a large red sow that had farrowed several litters of pigs. She came in heat on November 16, at 4 p. m., was bred at 9:30 a. m. on the 17th, and was killed at 4:30 p. m. of the same date. She was killed 33.5 hours after coming in heat and seven hours after being bred. An examination for sperm cells showed a very few slow-moving cells where the oviduct joins the horns of the uterus. Dead sperm cells were found in the lower half of the oviduct, also a few dead cells in the body of the uterus. The right ovary showed seven follicles and the left ovary five follicles, all ruptured.

Case No. 5. This sow was not in heat December 1, but was in heat on the morning of December 2. Time of coming in heat was estimated at 4 a. m. on December 2. This sow was bred at 9 a. m. on December 2, and killed at 9 a. m. on December 3. She was killed

29 hours after coming in heat and 24 hours after being bred. A careful examination failed to show any live sperm cells. A few dead cells were found in the horns of the uterus and in the lower portion of the oviduct. There were five well developed follicles in the right ovary and six in the left ovary, but none of them were ruptured.

Case No. 6. This sow came in heat at 7 a. m. on December 3, bred at 8 a. m., and killed at 6 p. m. on the same date. She was killed 11 hours after coming in heat and 10 hours after being bred. An examination showed an abundance of sperm cells at the junction of the oviduct and horn of uterus with approximately 2 to 5 per cent alive. Live sperm cells were also found along the horn of the uterus. There were three well developed follicles in the right ovary and six in the left ovary, but none of them were ruptured.

Case No. 7. This was a large sow that was considered as a non-breeder as she had been bred repeatedly without results. This sow was **not in heat** on December 2, but was bred at 7 a. m. on December 3. Time of coming in heat was estimated at 3 a. m. The sow was killed at 2:30 p. m. on the same date. She was killed 11.5 hours **after coming in heat** and 7.5 hours after being bred. Live sperm cells were found in the body of the uterus, in the uterine horns and in the lower third of the oviduct. There was not a great abundance of sperm cells found in any portion of the organs. Five to 10 per cent of the sperm cells were alive. A careful examination of the organs showed no abnormal condition except a small cyst that was found in the wall of the oviduct near the ovary on the right side. The ovaries appeared normal. There were 14 well developed follicles, none of them being ruptured.

Case No. 8. This sow came in heat at 2:30 p. m. on December 3, was bred at 7:30 a. m. December 4, and killed at 8:30 a. m. December 5. This sow was killed 42 hours after coming in heat and 25 hours after being bred. An examination of the ovaries showed 14 follicles with 13 of them ruptured. Ten yellow bodies (corpus luteum) were very prominent in the ovaries, showing where follicles had ruptured at the previous period of heat. These bodies are generally very distinct in the pig, but were unusually prominent in these ovaries. No live sperm cells could be found after a careful examination.

Case No. 9. This was a young sow, came in heat at 6 a. m. December 5, was bred at 4 p. m. on same date and killed at 9:30 a. m. December 7. This sow was killed 51.5 hours after coming in heat and 41.5 hours after being bred. The ovaries showed 12 ruptured follicles, seven in the right and five in the left ovary. No live sperm cells could be found in the oviducts or horns of uterus, but two live sperm cells were found in the body of the uterus. It will be noticed that in Cases 9 and 10 live sperm cells were found at a greater length of time after breeding than in any other cases of the experiment.

Case No. 10. This was a large white sow, a mate of No. 7, and was considered a non-breeder. She was killed 50.5 hours after coming in heat and 40.5 hours after being bred. No live sperm cells were found in the body or horns of the uterus, but a few live cells were found in the lower third of the oviduct. The ovaries showed 17 ruptured follicles, 10 in the left ovary and 7 in the right ovary. These follicles were small and appeared to be imperfectly developed. Otherwise no abnormal conditions could be found in any of the organs.

Case No. 11. This was a young sow that came in heat at 8 a. m. on December 8, was bred at 10:30 a. m., and killed at 2:30 p. m. on the same date. She was killed 6.5 hours after coming in heat and 4 hours after being bred. An examination showed sperm cells at all points along the uterus and horns of uterus, but no cells could be found in the oviducts. Probably 50 per cent of the cells found were alive. It will be noted that in this case within four hours after being bred the sperm cells were distributed the entire length of the uterus and uterine horns, a distance of more than three feet. The ovaries showed 15 well developed follicles, six in the left ovary and nine in the right ovary; none of them were ruptured.

Case No. 12. This sow came in heat at 12 p. m. on December 7, was bred at 9 a. m. December 8, and killed at 9 a. m. on December 9. She was killed 33 hours after coming in heat and 24 hours after being bred. This sow was not in heat on the afternoon of December 7, but was noticed to be in heat the next morning, and the time of coming in heat was approximated as 12 p. m. There were 17 ruptured follicles in the ovaries, ten in the left and eight in the right ovary. No live sperm cells could be found in any portion of the organs. A few dead cells could be found along the entire length of the uterine horns and in the lower portion of the oviduct.

Case No. 13. This sow came in heat at 6 a. m. on December 9 and was bred at 3 p. m. on the same date, and killed at 10 a. m. on December 10. She was killed 28 hours after coming in heat and 19 hours after being bred. The ovaries showed 13 fully matured follicles, eight in the right and five in the left ovary. None of the follicles were ruptured. No live sperm cells could be found in any portion of the organs, but dead sperm cells were found throughout the organs and in lower portion of oviducts.

Case No. 14. This sow was not bred, but was killed to see if any difference could be noted in the organs of the sow when not bred as compared with those that were bred. She was killed 32 hours after coming in heat. Post mortem examination showed both ovaries diseased. Several large cysts were present in each ovary, there being only one or two follicles that could be considered normal. The follicles that appeared normal were not ruptured. The ovaries from this case were photographed and are shown in Figure 1.

Case No. 15. This sow came in heat at 12 p. m. on December 9,was bred at 4 p. m. December 11, and killed at 10 a. m. on December 12. She was killed 58 hours after coming in heat and 18 hours after being bred. This sow was not in heat on the afternoon of December 9 and the time of coming in heat was approximated as 12 p. m. on that date. The ovaries showed 13 ruptured follicles, six in the right and seven in the left ovary. No live sperm cells could be found.

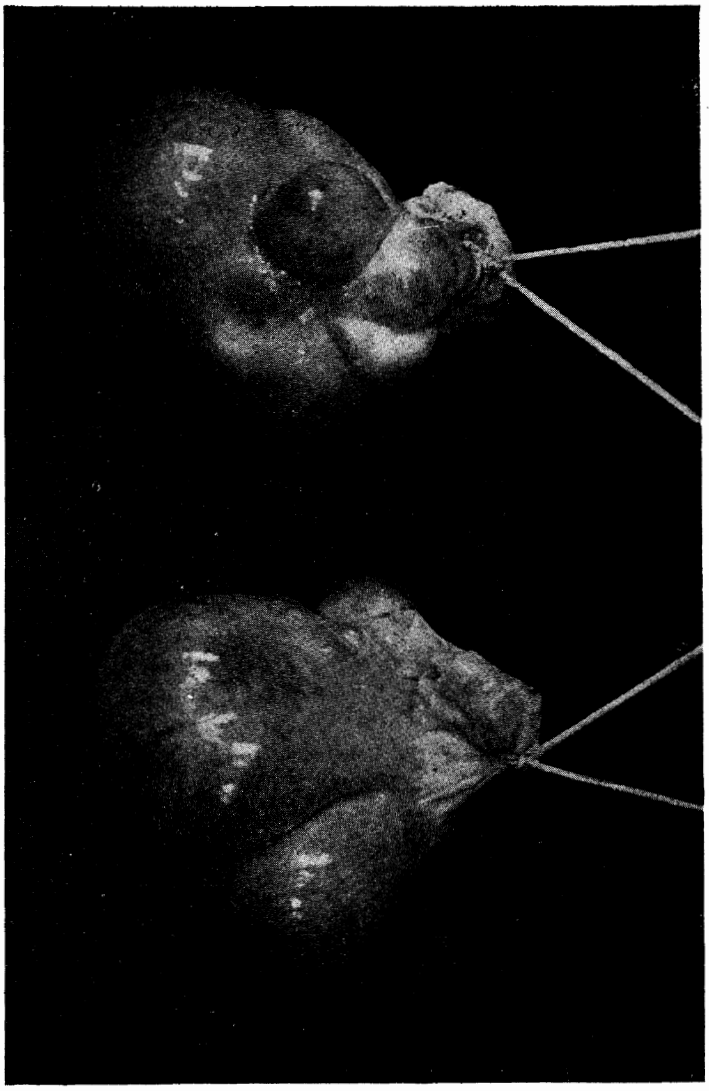


FIGURE 1

Diseased ovaries from Case No. 14. Large cysts were formed in each ovary.

Case No. 16. This sow came in heat at midnight of February 10, was bred at 1:30 on February 11, and killed at 9:30 a. m. on February 12. This sow was killed 33.5 hours after coming in heat and 20 hours after being bred. An examination of the ovaries showed 15 follicles, eleven of them being ruptured. The remaining follicles did

not appear fully matured as compared with the general appearance of the follicles during the period of heat. There were 4 follicles in the left ovary, all ruptured, 11 in the right ovary with 7 of them ruptured. No live sperm cells could be found in any portion of the reproductive organs.

Case No. 17. This sow came in heat at 4 a. m. on February 11, was bred at 1:30 p. m. on same date and killed at 10:30 a. m. on February 12. This sow was killed 30.5 hours after coming in heat and 21 hours after being bred. An examination of the ovaries showed that there was a large number of imperfectly developed follicles in each gland, probably as many as 50 in each ovary. None of these follicles were ruptured. No live sperm cells could be found in any portion of the reproductive organs.

Cases 18 and 19. These sows were not in heat and were not bred, but were butchered to determine the character of the secretions in the healthy organs under normal conditions. Immediately after the sows were killed the organs were removed and 250 cubic centimeters of distilled water was poured into each of the horns of the uterus, after which the tissue was manipulated so as to cause thorough washing of the mucous surface. The water was allowed to remain in the organs for five minutes. After handling the organs in the above manner the water was drawn into a beaker and tested to determine its reaction. With phenolphthalein as an indicator the washings from the organ of Case No. 18 required 1.8 cubic centimeters of tenth normal solution of sodium hydroxide to neutralize the acid contained in 25 cubic centimeters of the washings. Washings from the organs of Case No. 19 were tested in the same manner as described above and were found to be acid, but not quite to the same extent. Similar tests were made from Cases 20, 21 and 22 with the same general results, that is, the secretions were found to be acid to phenolphthalein.

Case No. 20. This sow came in heat at 12 p. m. February 27, was bred at 1 p. m. on February 28, and killed at 8 a. m. on March 1. This sow was killed 32 hours after coming in heat and 19 hours after being bred. The ovaries showed 12 matured follicles, none of them being ruptured. A careful examination failed to show any sperm cells in any portion of the organs.

Case No. 21. This sow came in heat at 10 a. m. on February 26, was bred at 4 p. m. on February 28, and killed at 8 a. m. on March 1. This sow was killed 70 hours after coming in heat and 16 hours after being bred. An examination of the ovaries showed 14 follicles, eight in the right and six in the left ovary. None of the follicles were ruptured. This case was peculiar on account of the great length of time after coming in heat before the Graffian follicles ruptured. After a lapse of 70 hours it would have been impossible for fertiliza-

tion to take place as the ovules had not escaped from the ovaries. The usual time, as may be seen by reference to the table at the end of this topic, is much shorter, varying to some extent but occurring most frequently at about 30 to 35 hours. A careful examination failed to show any sperm cells in the organs of this sow.

Case No. 22. This sow came in heat February 27 at 10 a. m., was bred at 4 p. m. on the same date, and killed at 3 p. m. on March 1. She was killed 53 hours after coming in heat and 47 hours after being bred. The ovaries showed 9 follicles, six in the right and 3 in the left ovary, all of them ruptured. A close examination of the organs failed to show any sperm cells. The washings made from the organs of this case, as described under Cases 18 and 19, were very acid.

Case No. 23. This sow came in heat at 4 a. m. on March 4, was bred at 2 p. m. on the same date, and was killed at 2 p. m. on March 5. She was killed 30 hours after coming in heat and 24 hours after being bred. The ovaries showed 12 follicles, the right ovary containing three follicles, two of them being ruptured, the left ovary containing nine follicles, with six of them ruptured. Examination failed to show any sperm cells in the organs.

Case No. 24. This sow came in heat at 12 m. on March 9, was bred at 11 a. m. on March 10, and killed at 5 p. m. on same date. This sow was killed 23 hours after coming in heat and 6 hours after being bred. The ovaries showed ten well developed follicles, six in the right ovary and four in the left ovary, none of them being ruptured. No sperm cells could be found in any portion of the organs.

Case No. 25. This sow came in heat at 12 p. m. on March 13, was bred at 8 a. m. on March 15, and killed at 12 m. on the same date. She was killed 36 hours after coming in heat and 4 hours after being bred. The ovaries showed 9 follicles, all of them ruptured. An examination showed only a few live sperm cells in the horns of the uterus.

Cases 21, 22, 23, 24 and 25. These sows were bred to a boar that was afterward tested as to vitality of sperm cells and was found to be a hog showing poor vitality or vigor so far as vitality of the sperm cells was concerned. Such an animal would probably prove utterly useless for breeding purposes. The data from these few cases is not reliable so far as the vitality of the sperm cells is concerned, but as to the other particulars regarding the time after coming in heat before the Graffian follicles of the ovary are found ruptured, the data is reliable.

The following table shows the important data obtained by breeding and killing the twenty-five sows just described:

No. of Case	Date	Hours from Time of Coming in Heat until Killed	Condition of the Ovaries When Killed	Hours from Time of Breeding Until Killed	Condition of Sperm Cells in Organs when Sow was Killed
	1908				
1	May 30	25.5	No follicles ruptured	16.	Found live cells
2	July 1	50.	Follicles ruptured	.....	Was not bred
3	" 10	46.5	No follicles ruptured	22.5	Found live cells
4	Nov. 17	24.5	Ten ruptured, two not	7.	Found live cells
5	Dec. 2	29.	No follicles ruptured	24.	No live sperm cells
6	" 3	11.	No follicles ruptured	10.	Found live cells
7	" 3	11.5	No follicles ruptured	7.5	Found live cells
8	" 5	42.	Follicles ruptured	25.	No live sperm cells
9	" 7	51.5	Follicles ruptured	41.5	Live sperm cells
10	" 7	50.5	Follicles ruptured	40.5	Live sperm cells
11	" 8	6.5	No follicles ruptured	4.	Live sperm cells
12	" 9	33.	Follicles ruptured	24.	No live sperm cells
13	" 10	28.	No follicles ruptured	19.	No live sperm cells
14	" 10	32.	No follicles ruptured	.....	Was not bred
15	" 12	58.	Follicles ruptured	18.	No live sperm cells
	1909				
16	Feb. 12	33.5	Eleven ruptured, 4 not	20.	No live sperm cells
17	" 12	30.5	Follicles not developed	21.	No live sperm cells
18	Was not in heat and was not bred				
19	Was not in heat and was not bred				
20	Mar. 1	32.	No follicles ruptured	16.	Live sperm cells
21	" 1	70.	No follicles ruptured	16.	No live sperm cells
22	" 1	53.	Follicles ruptured	47.	No live sperm cells
23	" 5	30.	Eight ruptured, 6 not	24.	No live sperm cells
24	" 10	29.	No follicles ruptured	6.	No live sperm cells
25	" 14	36.	Follicles ruptured	4.	Live sperm cells

Discussion

The total number of sows used in this experiment was twenty-five. The males used were two mature Berkshire boars. Semen was secured from these males and an examination showed that the vitality of the sperm cells was that of the average male hog. In only three cases in the experiments of 1908-1909 could live sperm cells be found at a greater length of time than twenty hours after breeding. In two cases live cells were found after a lapse of more than forty hours, and in one case after a lapse of twenty-two and one-half hours. Of the nineteen sows bred and killed, the sperm cells were found dead in 80 per cent of the cases examined where a period of sixteen hours or more had elapsed between the hour of service and the time when killed for examination. This estimate does not include those cases numbered from twenty-one to twenty-five, as they were bred to a boar that was afterward found to be lacking in vitality of semen.

It is not likely that ovulation occurs in hogs to any extent except when accompanied by the usual signs of heat. In all of the cases

killed during the period of heat where the sow was considered normal the ovaries showed that the usual physiological changes were taking place. If the hog was killed early in the period of heat the follicles showed perfect and unruptured, while those from hogs killed a few hours later showed the follicles ruptured. A few sows were killed when not in heat, and in none of these were the follicles found well developed or in condition to liberate the ovum, neither did they show any signs of the recent escape of the ovum from the organ. Variations from the normal no doubt do occur, but it is safe to assume that in the great majority of cases the follicles do not rupture before thirty hours after the period of heat begins. In one case the follicles were not ruptured after the lapse of more than seventy hours, and in another after a lapse of more than forty-five hours. As compared with the data obtained from the remainder of the cases examined these are unusual. In no case were the follicles found ruptured during the first twenty-four hours of heat, and in most of the cases a period of thirty hours elapsed after the first signs of heat before many of the egg cells escaped from the ovary.

By studying the notes and tables under this experiment it will be seen that as a rule certain conditions of the organs are found within rather narrow limits of time after the period of heat begins. In only one of the cases were the follicles found ruptured before a lapse of thirty hours after coming in heat.

Experiments in 1910-1911

In the experiments conducted during 1908-1909 the sows were bred at varying lengths of time after coming in heat and then killed from four to forty-seven hours later. In this experiment it was noted that the sperm cells did not live a great length of time and that in a comparatively short time all of the cells had disappeared. In order to get more data along this line some of the sows used in experiments in 1910 were bred and killed within a few hours after breeding. Sow No. 135 was bred out of heat and killed twenty-one hours later. An examination of the organs showed semen present in the uterine horns and an abundance of sperm cells. Those alive being approximately 10 per cent of the number present. On December 22, 1910, two sows, Nos. 343 and 350, were bred to different males. Sow No. 343 was in heat, and sow No. 350 was not in heat. These sows were killed twenty-four hours after they were bred, and the following facts observed at post mortem:

Sow No. 343: In heat when bred. An examination showed that practically all of the semen had been absorbed from the uterus and cornu. Scrapings from the mucous surface showed very few cells present with an occasional cell showing movement by a slight vibrating as turning of the head or nucleus.

Sow No. 350: Not in heat when bred. Post mortem examination showed the uterine cornu to contain a large amount of semen. A microscopic examination showed a large number of sperm cells present, and from 5 per cent to 10 per cent of the total number showing movement, some very active.

In a previous section of this bulletin the question of the vitality of the sperm cells in the body of the female is taken up fully as shown by results obtained on post mortem examination. All of the cases referred to in that section of the bulletin were where the breeding had been during the period of heat. In the work done during 1910-1911 sows No. 135 and 350 were bred out of heat and were killed twenty-one and twenty-four hours later for the purpose of securing some check on the former work. Examination of the organs showed a large number of sperm cells with approximately 10 per cent of them showing vitality. This result was so at variance with results obtained when sows were bred in heat that the following experiments were carried out:

Breeding Experiments 1910-1911

In this experiment it was planned to breed sows at varying lengths of time after going out of heat in order to learn if possible the length of time after ovulation that the ovum might be fertilized. It was assumed that if the ova were present and physiologically active that fertilization would occur. It was also planned that if no results followed breeding out of heat that before the sows were disposed of they would be bred either in or out of heat and killed at varying lengths of time afterward in order to not only obtain data to compare with that obtained as tabulated on page 14, but to determine the length of time live sperm cells could be found in the organs of a sow bred out of heat as compared with results obtained where sows were bred in heat.

The following data covers the essential points in the records of each of the sows used in this experiment:

Sow No. 130: August 27, in heat; August 30, not in heat; August 31, sow was bred; October 9, sow was in heat; October 11, not in heat; October 12, sow was bred; December 9, sow was killed and embryos found from breeding of October 12.

Sow No. 131: September 13, in heat; September 15, out of heat and bred; October 1 to 3, in heat; October 4, out of heat and bred; October 24 and 25, in heat; October 27 sow killed and organs examined. The Graffian follicles appeared to have ruptured only a few hours before the sow was killed.

Sow No. 132: August 17 to 21, in heat; August 23, out of heat and bred; September 27 to 29, the sow was in heat; October 4, the

sow was not in heat and was bred; November 7, the sow was not in heat; November 18, not in heat and bred; November 18, this sow was killed six and a half hours after breeding and an examination showed an abundance of the sperm cells, 30 per cent of them being alive. No results from previous breedings.

Sow No. 133: This sow was in heat August 16; September 16, not in heat and bred; September 27, in heat; September 29, not in heat and bred; October 17 and 18, sow was in heat; October 20, not in heat and bred; November 7 and 8, in heat; November 8, bred while in heat. November 25, sow was killed and embryos found from breeding of November 8.

Sow No. 134: September 10, sow was in heat; September 21, not in heat and bred; September 29-October 1, sow was in heat; October 7, sow was not in heat and bred; October 27, sow was killed. Post-mortem showed no results from breeding.

Sow No. 135: August 31, sow was in heat; September 3, out of heat; September 5, out of heat and bred; September 20, sow was in heat; September 21, out of heat; September 23, out of heat and bred; October 9, in heat; October 11, out of heat and bred; November 28, in heat; December 14, out of heat and bred at 5 p. m.; December 15, sow was killed at 2 p. m. and organs examined. A large number of live sperm cells were found. The organs were examined again at 5 p. m. and probably 5 per cent of the cells present showed vitality.

Sow No. 137: September 3 to 5, sow was in heat; September 10, sow was bred; September 23 to 27, sow was in heat; September 30, sow was out of heat and bred; October 15 to 17, sow was in heat; October 18, sow was out of heat and bred; November 5, sow was in heat and bred; November 8, sow was found dead in pen, and an examination showed no developing embryos from any breedings previous to November 5.

Sow No. 138: This sow farrowed pigs July 28, 1910; September 21 to 25, in heat; September 26, not in heat; September 27, sow was bred; October 17 to 20, sow was in heat; October 21, not in heat; October 22, sow was bred; November 9 to 11, in heat; December 3, in heat; December 24, in heat; December 28, out of heat; January 18, 1911, in heat; January 19, in heat and bred. Sow was killed at 2 p. m. on January 20, or 28 hours after breeding. Examination of the ovaries showed two follicles on right and six follicles on left ovary, all ruptured. A microscopic examination of the contents of the uterus showed a very few sperm cells, but approximately 30 per cent of those present were alive. The movement was not active, but consisted in a slight vibrating or turning of the head of the sperm.

Sow No. 141: This sow was in heat August 31, was out of heat and bred on September 5; September 23, in heat and was bred; Sep-

tember 25, out of heat and bred; October 11 to 13, was in heat, and bred out of heat on October 15. This sow was bred on November 3, which was the second day of this period of heat. On November 22 this sow was again bred, but on the third day of heat. On December 7 this sow was killed and six embryos found from breeding of November 22.

Sow No. 142: August 27, sow in heat; August 29, not in heat and bred; September 13 to 16, sow in heat; September 20, sow out of heat and bred; October 22 to 24, sow in heat; October 25, not in heat and bred; November 8, sow in heat; November 9, in heat and bred; November 30, sow was killed and embryos found from the breeding of November 9.

Sow No. 143: July 26, sow was in heat; August 5, not in heat and bred; August 24, in heat; August 28, not in heat and bred; September 15 and 16, in heat; September 20, not in heat and bred; October 7 to 10, in heat; October 11, not in heat and bred; December 7, sow was killed and an examination showed developing embryos from breeding of October 11.

Sow No. 144: September 10, sow in heat; September 16, out of heat and bred; September 29, in heat; September 30, sow out of heat; October 1, out of heat and bred; October 20, sow in heat; October 22, sow out of heat and bred; November 10, sow in heat; November 11, sow in heat and bred; November 30, sow was killed and embryos found from breeding of November 11.

Sow No. 145: August 16, sow was in heat; September 5, not in heat but bred; September 27 to 29, sow was in heat; October 3, not in heat but bred; October 28, killed and found to be not bred.

Sow No. 336: This sow was in heat on January 28, 1911, and was bred while in heat on January 30 at 3:00 p. m. This sow was killed 24 hours later and a careful examination showed no live sperm cells and no free semen in the organs. A very few dead sperm cells were found in making scrapings from the membranes of the cornu. Seven Graffian follicles were noticed, all ruptured.

Sow No. 348: This sow was bred at 3 p. m. on the third day of the period of heat and was killed twenty-five hours afterward in order to learn the condition of the organs. Examination showed all of the Graffian follicles ruptured. A microscopic examination of scrapings from the body of the uterus showed a considerable number of sperm cells and probably 25 per cent of these showed vitality by slow vibrating movements.

Sow No. 349: This sow was bred out of heat on March 21, 1911, and killed forty-eight hours afterward. A microscopic examination showed some sperm cells in uterine cornu, but none were found alive.

After these sows were bred a few times out of heat, and no results obtained, it was decided to breed most of them during a period of heat, as in most cases there was no data to show that they were breeders. Sow No. 135 was bred the last time out of heat, and then killed in order to get additional data regarding the length of time the sperm cells will live within the body of the female.

Table

The following tabulated statement will show the results obtained when sows were bred out of heat:

No.	Times Bred	Results of Breeding	Time After Heat Before Bred
130	Bred twice out of heat	Breeding successful	One day in each case
131	Bred twice out of heat	No results from breeding	Two and one days
132	Bred twice out of heat	No results from breeding	Two and five days
133	Bred 3 times out of heat	No results from breeding	Thirty, 2 and 2 days
134	Bred twice out of heat	No results from breeding	Seven and ten days
135	Bred 3 times out of heat	No results from breeding	Five, 3 and 2 days
137	Bred 3 times out of heat	No results from breeding	One, 3 and 5 days
138	Bred twice out of heat	No results from breeding	One and two days
141	Bred 3 times out of heat	No results from breeding	Five, 2 and 2 days
142	Bred 3 times out of heat	No results from breeding	Two, 4 and 1 days
143	Bred 4 times out of heat	Breeding successful	Ten, 4, 4 and 1 days
144	Bred 3 times out of heat	No results from breeding	Six, 2 and 2 days
145	Bred twice out of heat	No results from breeding	Twenty and 5 days

VITALITY OF THE EGG CELL OR OVUM

It seems quite as important to know something of the general vitality of the egg cell as it is to know the vitality of the sperm cell. A study of the vitality of the egg cell is not as easily made as is that of the sperm, yet the results of the limited number of experiments conducted seem to indicate that from a physiological standpoint the vitality of the ovum or egg may be determined quite definitely.

To determine this question sows were bred at varying lengths of time after the period of heat had passed (see records of Sows Nos. 130-145), as it was thought that the ova would be fertilized if they were in such physiological condition as to be capable of fertilization. Previous observations had shown that the ova are not liberated from the ovaries during the early period of heat, but are liberated during the latter part of the period of heat. A number of sows were used in this experiment. Records were kept as to the time the period of heat began, and in nearly all cases the date was noted when the period of heat ended. By breeding at different lengths of time after the period of heat ended very good evidence was obtained regarding the length of time the egg cell is capable of being fertilized after it leaves the ovary.

By referring to condensed records of the hogs used in this test, (as shown on pages 16-21), some information may be obtained relative to the vitality of the ovum. Data is given covering the experiments with thirteen sows that were bred out of heat. Thirty-four services were given these hogs while out of heat; seven services were given on the first day after the period of heat had passed, with successful results following in only two cases. If, as previous observations seem to show, the egg cell is not liberated from the ovary until near the close of the period of heat, and more than 70 per cent of the breeding is without results when service is given within twenty-four hours after the period of heat, and no results follow from breeding at a later day, it would seem that the ovum or egg does not retain its vitality for more than a few hours after being liberated from the Graffian follicles.

By referring to the detailed record of the hogs used in 1910-1911 experiments, very good evidence is obtained that the sperm cell will not live for a great length of time in the body of the female. Many of the sows in this experiment were bred out of heat and observations continued in some cases for more than four months, and in no case was there any result from breeding out of heat where more than twenty-four hours elapsed after the period of heat. If the sperm cell will live for two or three weeks, then fertilization of the ova should occur at the first period of heat following the breeding. The time intervening from the time the sow was bred out of heat until she was in heat again varied in the several cases from seven to twenty-two days. It may be that in drawing conclusions in regard to this work that factors are to be considered that we have no intimate knowledge of, yet the observations made on post mortem examinations and results following breeding out of heat indicate that the ovum and sperm cell soon lose their physiological activity after they became separated from the ovary and testes.

VITALITY OF SEMEN UNDER LABORATORY CONDITIONS

In connection with the series of experiments just reported, in which the data seems to show that the vitality of the sperm cell of the hog under normal breeding conditions is relatively short, it will be of interest to give the data connected with observations on the vitality of the semen under laboratory conditions.

On July 7, 1907, semen was collected from a young Poland China boar, a vigorous and well developed animal. The semen was collected at 7:30 a. m. and kept at 30° C. An examination was made within a few minutes after the semen was collected and practically all of the sperm cells were active. Observations were made as follows:

7:30 a. m. ..... 100 per cent of the cells alive
10:30 a. m. ..... 75-85 per cent of the cells alive
2:00 p. m. ..... 25-35 per cent of the cells alive
5:00 p. m. ..... 20-25 per cent of the cells alive
9:00 p. m. ..... 5 per cent of the cells alive

The number of sperm cells was determined, the average of two counts giving 365,520 sperm cells per cubic millimeter.

On July 9, 1907, a sample of semen was secured from a Hampshire boar that was an unusually vigorous animal. Portions of this semen were kept at different temperatures and observations made at intervals to determine the effect of various temperatures on vitality. The semen was obtained at 10:00 a. m. The number of sperm cells per cubic millimeter was 384,500.

Time.	Semen at 22° C.	Semen at 30° C.
10:00 a. m.	100 per cent	100 per cent
12:00 m.	90 per cent	85 per cent
2:30 p. m.	80 per cent	50 per cent
5:00 p. m.	55 per cent	35 per cent
10:00 p. m.	30 per cent	5 per cent
7:00 a. m. July 10	5 per cent	
10:00 a. m.	1 per cent	

The sperm cells of the semen in this test retained their vitality longer than cells from any other animal that had been tested in connection with this work up to this time. Other tests made of semen from this boar also indicated exceptional vitality of the cells.

On July 19, 1907, about two ounces of semen was secured from a young Berkshire boar at 7:30 a. m. This was examined within a few minutes after it was collected and practically all of the cells were found to be active. The semen was kept at 30° C. and away from direct sunlight, but in the diffused light of the room:

7:30 a. m. ..... 100 per cent of the cells alive
4:00 p. m. ..... 65-75 per cent of the cells alive
8:00 p. m. ..... 20-30 per cent of the cells alive
10:00 p. m. ..... No movement noticed except in an occasional cell.

On July 19, 1907, semen was collected from boar No. 466 (matured Poland China) at 7:30 a. m. Semen was kept at 30° C.

7:30 a. m. ..... 100 per cent of the cells alive
10:00 a. m. ..... 75-85 per cent of the cells alive
4:00 p. m. ..... 50-60 per cent of the cells alive
8:00 p. m. ..... 20 per cent of the cells alive
10:00 p. m. ..... No live cells noted.

From the above experiments it will be seen that under experimental conditions the vitality of the sperm cells from the boar continues approximately fifteen to twenty-five hours after the semen is collected. The length of time depending upon the temperature at which the semen is kept and the vigor and physical condition of the animal used in the experiment. Temperature has much the same effect on the vitality of the semen from the hog as it has on that collected from the horse.

The following experiment serves to illustrate the marked effect of temperature on vitality of the sperm cells from the hog:

On July 11, 1908, a mature Berkshire boar was bred at 8:30 a. m. and approximately ninety cubic centimeters of semen secured. This was divided into a number of lots of twenty cubic centimeters, each being kept under slightly different conditions.

Lot A was kept in the incubator at a temperature of 66° C.

Lot B was kept on top of the incubator at a temperature of 31°-32° C. This lot was divided so that one portion was completely protected from the light by wrapping bottle in black paper.

Lot C was kept in an ice box at a temperature of 22° C. This lot was divided so that one portion was completely protected from any light.

Lot D was placed in the open window in direct sunlight at a temperature of 31° C. This lot was divided so that one portion was completely protected from the sunlight, but was exposed to same temperature:

Time.	Lot A.	Lot B.	Lot C.	Lot D.
10:00 a. m.	90 per cent	95 per cent	90 per cent	10 per cent
11:00 a. m.	85 per cent	80 per cent	90 per cent	0 per cent
1:00 p. m.	15 per cent	60 per cent	75 per cent	
3:30 p. m.	0 per cent	1 per cent	25 per cent	

It required approximately thirty minutes to reach the laboratory and distribute the semen as above indicated, therefore the time of exposure to the above temperatures began at 9 o'clock. In Lot B the portion that was wrapped in black paper showed 5 per cent alive at 3:30. In lot C the portion protected from the light was not disturbed until 3:30 p. m., when the lot showed approximately 40 per cent alive, although the movements were very slow. In Lot D the portion exposed to direct sunlight was killed at 10:30, but no observation was made on any of the other lots at that time, while the portion protected from direct sunlight showed 80 to 90 per cent alive at that time. Some of this protected sample was exposed to the direct sunlight at 10:30 while the remainder was again protected from light. The portion exposed showed no live cells at 11:30 while the unexposed portion showed 25 per cent alive at 1 p. m. At that time the temperature had

risen to 35° C. The direct sunlight is quickly fatal to the sperm cells and diffused light is harmful to some extent, although in the ordinary light of the laboratory where the samples were kept, fifteen to twenty feet from the window, it seemed to have very little effect.

Semen from different males varies greatly in its vitality under laboratory conditions. This difference is sometimes so noticeable as to indicate the probability of poor breeding qualities.

As a means of comparison, the following data is introduced as the animals used were all different from those used in the experiments of 1907 and 1908:

Hog No. 342: This is a young Berkshire boar, weight 209 pounds. Semen was collected at 2 p. m. on February 4, 1911, and placed immediately in test.

Time.	Temp.	per cent. alive.	Temp.	per cent. alive.	Temp.	per cent. alive.
2:00 p. m.	37° C.	98	26° C.	94	20° C.	96
4:00 p. m.	37° C.	82	26° C.	80	20° C.	87
5:00 p. m.	37° C.	80	26° C.	75	20° C.	80
7:30 p. m.	37° C.	60	24° C.	70	20° C.	75
8:30 a. m.	35° C.	0	22° C.	0	16° C.	30
2:00 p. m.					20° C.	15
6:30 p. m.					20° C.	08

Number of sperm cells per cubic millimeter, 600,000.

Hog No. 344: Mature Duroc boar. Semen was secured at 10:30 a .m. on February 21, 1911. This material reached the laboratory at body temperature and was divided into three lots, as indicated in the following table. All of the material for the tests was handled in the same manner except that the temperature at which the samples were kept varied a few degrees:

Time.	Temp.	per cent. alive.	Temp.	per cent. alive.	Temp.	per cent. alive.
February 27, 1911.						
10:30 a. m.	35° C.	100	23° C.	100	15° C.	100
11:30 a. m.	33° C.	98	25° C.	100	15° C.	100
1:30 p. m.	32° C.	95	27° C.	96	15° C.	98
3:30 p. m.	33° C.	88	27° C.	90	16° C.	93
5:30 p. m.	34° C.	75	28° C.	86	15° C.	88
7:30 p. m.	33° C.	70	28° C.	85	23° C.	70
10:30 p. m.	32° C.	70	28° C.	70	23° C.	65
8:00 a. m.	33° C.	0	21° C.	15	15° C.	15
10:30 a. m.	33° C.	0	25° C.	15	15° C.	15
3:00 p. m.			28° C.	4	15° C.	5
5:15 a. m.			28° C.	0	15° C.	2

Number of sperm cells per cubic millimeter, 427,000.

Hog No. 345: This is a young Poland China boar, weight 227 pounds. Semen was secured at 10:45 on March 3, 1911, and placed in laboratory test at 11 a. m.

Time.	Temp.	per cent. alive.	Temp.	per cent. alive.	Temp.	per cent. alive.
11:00 a. m.	34° C.	100	23° C.	100	18° C.	100
12:00 m.	34° C.	98	23° C.	100	18° C.	100
1:30 p. m.	34° C.	96	23° C.	97	18° C.	99
3:00 p. m.	35° C.	95	27° C.	95	20° C.	98
6:00 p. m.	35° C.	90	25° C.	93	20° C.	95
7:15 p. m.	35° C.	90	25° C.	93	20° C.	93
9:30 p. m.	33° C.	87	24° C.	90	19° C.	90
March 4.						
7:30 a. m.	30° C.	0	22° C.	75	11° C.	60
11:30 a. m.	36° C.	0	28° C.	60	18° C.	55
2:00 p. m.	36° C.	0	26° C.	55	20° C.	50
5:00 p. m.	36° C.	0	25° C.	40	20° C.	45
March 5.						
8:55 a. m.	36° C.	0	25° C.	1	15° C.	3
11:00 a. m.	36° C.	0	24° C.	0	16° C.	1
2:30 p. m.	36° C.	0	24° C.	0	18° C.	1

Number of sperm cells per cubic millimeter, 518,000.

Hog No. 340: This is a young Duroc boar, weight 195 pounds. The semen was collected at 10:45 a. m. on March 9th, 1911, and reached the laboratory by 11 o'clock.

Time.	Temp.	per cent. alive.	Temp.	per cent. alive.	Temp.	per cent. alive.
11:00 a. m.	36° C.	100	28° C.	100	22° C.	100
1:30 p. m.	38° C.	93	32° C.	95	23° C.	95
3:30 p. m.	38° C.	87	32° C.	89	24° C.	90
5:30 p. m.	37° C.	1	29° C.	75	24° C.	80
9:30 p. m.			27° C.	60	20° C.	70
March 10.						
8:00 a. m.			25° C.	5	20° C.	25
11:30 a. m.			27° C.	1	21° C.	15
2:00 p. m.					21° C.	5
4:00 p. m.					21° C.	0

Number of sperm cells per cubic millimeter was 302,000.

THE EFFECT OF CONTINUOUS SERVICE ON THE NUMBER AND VITALITY OF SPERM CELLS

It is of consnderable importance to know the effect of continuous breeding on the vitality and number of sperm cells, and particularly

to know the effect upon the vitality of the cells. Some breeders make a practice of crowding a stallion or jack with services, often with poor returns in the matter of a very low per cent of foals. The effect of frequent service is, however, a matter in which the individuality of the horse plays a very great part. The number and vitality of the sperm cells may be reduced in the semen from one horse by five or six services as much as it would be in another individual by two weeks' continuous service. Some idea of this general effect may be drawn from the general temperament of the animal, but this cannot always hold good.

In order to secure some data along this particular line use was made of two stallions owned by the College. One of the horses is of a heavy draft breed, six years old and of a sluggish temperament. The other horse is a grade Shire stallion, five years old and is a very vigorous and well developed horse. He is not of the nervous temperament often seen in the light breeds of horses, but is not to be considered of a sluggish disposition. These horses were used not only in this test, but were used in other experiments connected with this work. The grade stallion has been used for most of the work reported in this bulletin where the vitality of the semen from the horse was studied. The general plan of the experiment was to breed the stallion daily and to secure samples of the semen for laboratory work in order to make a count of the number of sperm cells present as well as to determine the length of time they would retain their vitality under laboratory conditions. Only the data of table No. 1 is from material obtained from the draft stallion, the remainder of the work reported was carried out with semen from the lighter or grade horse.

In preparing the semen so as to make a count of the cells present, the following procedure was used:
From the entire lot of semen that was collected, one cubic centimeter was taken and added to 24 cubic centimeters of a 2 per cent solution of sodium carbonate. The apparatus used for counting the sperm cells was the Thoma-Zeiss Haemacytometer. Usually two hundred squares were counted in each preparation, and as a rule two preparations were made from each dilution. After using a number of different solutions for diluting the semen the carbonate solution was finally selected as giving the best results, as it prevented the clumping of the sperm cells.

Experiment No. 1. The draft stallion was used in this experiment and was bred daily for nine consecutive days. The semen was collected either from the vagina or from the horse as he dismounted, as he would not serve with the breeding bag. Usually the cervical plug was used to prevent the entrance of the semen into the womb. The semen from each service was taken to the laboratory in a clean glass vessel and kept near body temperature by means of warm water.

The vitality of the sperm cells was determined for each sample under laboratory conditions as well as a count made to determine the number of cubic millimeters in each sample. As a means of comparing the vitality of the sperm cells from different services of the test, the laboratory data will be given for material obtained at three of the services.

First service in the test, February 13, 1908. Secured the semen at 8:30 a. m. and it was placed immediately in a clean warm glass vessel and reached the laboratory within a few minutes at a temperature of 38° C. The semen was divided into two lots, one lot was kept at a temperature of 33° C., and the other at a temperature of 24° C. Microscopic examinations were made at certain intervals of time with the following results:

Time.	Lot kept at 33° C.	Lot kept at 24° C.
9:30 a. m.	Practically all alive.	Practically all alive.
11:00 a. m.	60 to 70 per cent alive.	70 per cent alive.
1:00 p. m.	40 per cent alive.	55 per cent alive.
3:30 p. m.	10 per cent alive.	45 per cent alive.
6:00 p. m.	Less than 5 per cent alive.	20 per cent alive.

Number of sperm cells per cubic millimeter was 131,750.

Fourth service of the test, February 16, 1908. The semen was secured at 2:30 p. m. and reached the laboratory within a few minutes at a temperature of 36° C. The semen was divided immediately into two lots; one was kept at a temperature of 31° C. and the other at a temperature of 23° C.

Time.	Lot kept at 31° C.	Lot kept at 23° C.
3:00 p. m.	Practically all alive.	Practically all alive.
4:00 p. m.	75 per cent of cells alive.	80 per cent of cells alive.
8:00 p. m.	10 per cent alive.	40 per cent alive.
10:00 p. m.	Cells all dead.	10 per cent alive.

The number of sperm cells per cubic millimeter was 78,000.

Sixth service of the test, February 18, 1908. The principal reason for giving the laboratory record of this material is on account of the great variation of temperature to which some of the semen was exposed. As soon as the semen was collected it was divided into equal parts. One portion was kept warm and reached the laboratory at a temperature of 35° C. The second portion was placed in an open glass vessel and had a temperature of 16° C.. when it reached the laboratory. Immediately after the semen reached the laboratory each lot was divided into two portions, each portion of the same lot being placed at different temperatures:

		Kept at 34° C. in Laboratory	Kept at 23° C. in Laboratory
Part that reached Laboratory at 35° C.	4 p. m.	35 per cent alive	50 per cent alive
	6 p. m.	15 per cent alive	30 per cent alive
	7 p. m.	5 per cent alive	20 per cent alive
	8 p. m.	Cells all dead	5 per cent alive
Part that reached Laboratory at 16° C.	4 p. m.	25 per cent alive	20 per cent alive
	6 p. m.	10 per cent alive	15 per cent alive
	7 p. m.	Cells all dead	5 per cent alive
	8 p. m,	.....	Cells all dead

The number of sperm cells per cubic millimeter was 54,280.

Both lots of semen were examined as soon as they reached the laboratory and practically all of the cells were alive. By noting the above records it will be seen that the temperature of the semen was reduced from that of the body, or about 38° C. to 16° C. within a few minutes, and a portion of the material was again raised to 34° C. in a short time, the remainder being raised to 23° C. The exposure of the semen to this wide variation of temperature did not have as marked an effect as might have been expected. Other experiments where the temperature was varied as in the above as well as where a continuous low temperature was used indicates that the semen is not very sensitive to variations in temperature so far as microscopic examinations are concerned. Chilling the semen soon after it is collected does not appear to destroy its vitality to any considerable extent. Neither does the exposure of the semen to the atmosphere seem to influence the vitality of the cells. In some of the experiments the semen was removed as soon as possible from the breeding bag and placed in sterile glass tubes covered with black paper. These tubes were kept sealed and excluded from light. Under these conditions the vitality of the semen was not prolonged much beyond that of semen from the same service kept in open vessels in the laboratory but under the same conditions as to temperature. So far as present knowledge of the fertilizing power of the sperm cell is concerned it cannot be said that because neither chilling, nor exposure to air, seems to influence vitality as determined by the microscope, that these conditions have no harmful effect on the normal function or fertilizing power of the cell.

Table I

The following table summarizes the results obtained with the draft stallion in this test. He was bred each day, as shown by the table, the semen was collected either from the horse or from vagina of the mare, was carried to the laboratory in a clean warm glass vessel and examined immediately to determine the general condition as to vitality.

Date and Time of Service	Time Semen Lived at 21-23° C.	Time Semen Lived at 31-35° C.	No. of Sperm Cells per Cubic Millimeter
Feb. 13, 1908 8:30 a. m.	20 per cent alive after 9.5 hours	5 per cent alive after 9.5 hours	131,750
Feb. 14, 1908 9:00 a. m.	No sample of semen obtained	No sample of semen obtained	
Feb. 15, 1908 8:30 a. m.	5 per cent alive after 9.5 hours	No cells alive after 6 hours	100,625
Feb. 16, 1908 2:30 p. m.	10 per cent alive after 7.5 hours	10 per cent alive after 5.5 hours	78,000
Feb. 17, 1908 9:00 a. m.	No cells alive after 9 hours	No cells alive after 6.5 hours	97,875
Feb. 18, 1908 3:00 p. m.	5 per cent alive after 5 hours	No cells alive after 4 hours	54,280
Feb. 19, 1908 2:00 p. m.	2 per cent alive after 4 hours	No cells alive after 3 hours	53,780
Feb. 20, 1908 9:00 a. m.	No cells alive after 4 hours	No cells alive after 3 hours	51,480
Feb. 21, 1908 8:45 a. m.	No cells alive after 4.5 hours		5,840

The number of sperm cells in the semen rapidly decreased from 131,750 per cubic millimeter to 5,840 per cubic millimeter in nine services. The quantity collected at the first service of the test was 65 cubic centimeters (about 2 ounces), while at the last service all that could be collected was 5 c. c. The horse was not bred again until March 11, when 60 c. c. of semen was collected. The laboratory notes kept of this material show that 5 per cent of the sperm cells were alive after seven hours when kept at 30° C. and that 15 per cent were alive after 8.5 hours in the lot kept at 22° C. The semen had 127,625 sperm cells per cubic millimeter. It is not likely that all of the time from February 21, the last day of the test, until March 11, the first service made by the horse after the test, was required for the semen to regain its normal condition as to number and vitality of the sperm cells. After continuous service, when the number and vitality of the sperm cells have fallen below normal, the return to normal conditions is noted withen seven to ten days and in some cases less time was required.

Experiment No. 2. The grade stallion was used in this experiment. This horse did not object to the use of a breeding bag, consequently the semen was collected in this manner, making the work

much more reliable in certain particulars than in the previous test. Samples of the semen from each service were tested under laboratory conditions at different temperatures, but detailed notes will be given only for the first, sixth and tenth services of the test. Eleven consecutive services were made in this test.

First service, March 30, 1908. The semen was collected at 11:30 a. m. and reached the laboratory within a few minutes at a temperature of 38° C. This lot was kept at only one temperature, 23° C.:

11:30 a. m.	Practically all of the cells alive.
1:00 p. m.	80 per cent of the cells alive.
2:00 p. m.	60 per cent of the cells alive.
4:00 p. m.	35 per cent of the cells alive.
6:00 p. m.	20 per cent of the cells alive.
8:00 p. m.	5 per cent of the cells alive.

When the observation at 4 p. m. was made the vessel containing the semen was by mistake placed in an incubator having a temperature of 27° C. This probably interfered to some extent with the length of time the cells lived, as the semen invariably loses its vitality quicker at a higher temperature. The number of sperm cells in a cubic millimeter was 232,500.

Sixth service of the test, April 3, 1908. The semen was obtained at 10:30 a. m. and reached the laboratory within a few minutes at a temperature of 38° C. A microscopic examination showed practically all of the cells alive and very active. Four ounces of semen were collected at this service. The semen from this service was divided into six lots, each lot being placed in clean glass beakers warmed to 35° C., after which the beakers were placed under different conditions, either with reference to temperature or to light.

Time.	Lot 1. 23° C.	Lot 2. 30° C.	Lot 3. 18° C.	Lot 4. 20° C.	Lot 5. 23° C.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
10:30 a. m.	100	100	100	100	100
11:30 a. m.	90	90	95	90	X
1:30 p. m.	50	10	75	20	X
3:00 p. m.	10	dead	40	dead	40
3:30 p. m.	5		20		15

Lot 1 was kept in the diffused light of the laboratory, Lot 2 was the same as Lot 1, with the difference in temperature; Lot 3 was kept in the dark room and was not exposed to light except when material was secured for microscopic examination. The lot placed in the dark room was checked by a lot kept in the laboratory in diffused light, but at the same temperature of Lot 3. At 3 p. m. the check lot

showed approximately 35 per cent alive, while the lot in the dark room showed approximately 40 per cent alive. Lot 4 was placed in direct sunlight in the window, while Lot 5 was placed in a gelatin capsule, but kept at the same temperature of Lot 1. The semen contained 60,500 sperm cells per cubic millimeter. Temperature seems to exert a greater influence upon the vitality of the semen than any other condition entering into the experiment. Direct sunlight has a marked harmful effect as noted in the above test. This effect of sunlight has been confirmed in a number of other tests which will be referred to again in another portion of the bulletin. The semen kept at the lowest temperature, 18° C., showed a much higher degree of vitality than that kept at a higher temperature.

Tenth service of the test, April 7, 1908. The semen was obtained at 10:30 p. m. and reached the laboratory within a few minutes after it was secured, at a temperature of 37° C. A microscopic examination showed practically all of the cells active. The semen from the last few services appears to be much thinner than semen under ordinary conditions. Eighty c. c. were collected and divided into three lots as follows:

Time	Lot 1—22° C.	Lot 2—35° C.	Lot 3—22° C.
1:30	All cells active	All cells active	All cells active
3:00	70 per cent alive	40 per cent alive	60 per cent alive
4:30	40 per cent alive	15 per cent alive	30 per cent alive
5.15	10 per cent alive	Cells dead	5 per cent alive

Lot 3 was kept under the same conditions as Lot 1, but had five drops of sodium citrate solution (4 per cent solution) added to each 6 c. c. of the semen. This amount of the chemical had only a very slight effect upon vitality. In a number of other experiments small amounts of different chemicals were added to small quantities of semen and practically all of the materials tested lessened the vitality of the sperm cells, and most of the chemicals appear to act as direct poisons, stopping the movement within a few minutes. There were 47,625 sperm cells per cubic millimeter. A brief calculation will show that there were present in the semen from this one service nearly four billion sperm cells. The semen tested slightly acid to phenolphthalin.

Table II

This table shows the summary of results obtained by examining the semen from each of the eleven consecutive services:

Date and Time of Service 1908	Time Semen Lived When Kept at 22-25° C,	Time Semen Lived When Kept at 32-35° C.	No. of Sperm Cells per cu. mm.
March 30 11:30 a. m.	5 per cent alive after 8.5 hours		232,500
March 31 8 a. m.	No semen secured		
March 31* 1:30 p. m.	5 per cent alive after 5 hours	All dead after 5 hours	83,000
April 1 10:30 a. m.	Urine was present in the sample		
April 2 9:15 a. m.	Cells dead after 8 hours	Cells dead after 6 hours	132,500
April 3 10:30 a. m.	5 per cent alive after 5 hours	Cells dead after 4.5 hours	60,500
April 4 8 a. m.	5 per cent alive after 7 hours	Cells dead after 5.5 hours	110,400
April 5 9:15 a. m.	40 per cent alive after 6 hours	Cells dead after 6 hours	72,000
April 6 8:45 a. m.	10 per cent alive after 8 hours	5 per cent alive after 6 hours	62,875
April 7 1:30 p. m.	10 per cent alive after 3.75 hours	Cells dead after 3:75 hours	47,625
April 8 10:15 a. m.	Cells dead after 5 hours	Cells dead after 4.5 hours	43,000

* This was the second service for this date, services 5.5 hours apart. Urine was probably present in the sample which would account for the short length of time the sperm cells lived.

A breeding bag was used in all of the services of this experiment and all of the samples were treated in the same manner as far as conveying the material from the barn to the laboratory is concerned. The semen was poured from the breeding bag into a clean and warmed glass vessel which was kept warmed by being partially immersed in warm water and covered by a heavy cloth. By noting the number of sperm cells present and the length of time they lived it will be seen that apparently the greatest difference is to be found at the beginning of the period of service. The first service gave cells alive after 8.5 hours that were kept at 23° C., while sample of the

semen from the third service made on the afternoon of the second day showed but few cells alive after five hours. The number of sperm cells per cubic millimeter also decreased from 232,000 in the first service to 83,000 at the third service. Approximately the vitality of the cells decreased one-half and the number to one-fifth in the last or eleventh service of the series as compared with the condition of the semen at the first service.

Experiment No. 3. This experiment will be given briefly as a means of comparing the condition of the semen with the results obtained from the first test with this stallion. In this experiment the services were begun on October 27 and continued daily for nine services, then two services were given daily until a total of eighteen services were had. The semen from a number of the services was taken to the laboratory and divided into lots that were run at different temperature in order to determine the vitality of the sperm cells. In every service from which semen was secured counts were made to determine the number of sperm cells present in a given amount of the semen (one cubic millimeter).

Date and Time of Service, 1908	No. of Service	Semen Kept at 32-35° C.	Semen Kept at 13-21° C.	No. of Sperm Cells
Oct. 27 9:30 a. m.	1	10 per cent alive after 6.5 hours	25 per cent alive after 6.5 hours	428,000
Oct. 30 9:30 a. m.	4	2 per cent alive after 4 hours	30 per cent alive after 7 hours	153,500
Nov. 2 9:30 a. m.	7		5 per cent alive after 5 hours	95,650
Nov. 4 9:30 a. m.	9	10 per cent alive after 3 hours	15 per cent alive after 6 hours	103,750
Nov. 6 9:15 a. m.	12	2 per cent alive after 3.5 hours	5 per cent alive after 7.5 hours	85,000
Nov. 8 5:30 p. m.	17		5 per cent alive after 6 hours	74,300

The horse was not bred again until November 14, when two ounces of semen was secured, and then again on November 20 when three ounces of semen was obtained. Data from these two services was secured in order to determine the length of time it would require for the semen to again reach the normal condition as shown in the first services. The material obtained on November 14 was divided into four lots as follows:

	Lot 1. Temp. 33° C.	Lot 2. Temp. 39° C.	Lot 3. Temp. 18° C.	Lot 4. Temp. 26° C.
10:45 a. m.	All alive.	All alive.	All alive.	All alive.
12:00 m.	40 per cent.	45 per cent.	90 per cent.	85 per cent.
1:00 p: m.	2 per cent.	2 per cent.	80 per cent.	65 per cent.
2:30 p. m.	dead.	dead.	65 per cent.	45 per cent.
4:00 p. m.			40 per cent.	20 per cent.
5:00 p. m.			30 per cent.	10 per cent.
7:30 p. m.			20 per cent.	2 per cent.

Lot 1 was exposed to bright sunlight. Lot 2 was kept in a dark incubator, and Lots 3 and 4 were in diffused light. It will be seen that approximately five days elapsed between the time of the service on November 8 and the service of November 14, and yet the number of cells had not increased to any extent, being practically lower than in any of the tests during the series of services. The next service of the stallion was on November 20, or six days later. The results of this service are given briefly in the following table. A count made showed 225,000 sperm cells per cubic millimeter, or a total of more than twenty billion of cells from this one service. The semen (90 c. c.) was divided into five lots, but only two temperatures were used.

Lot 1 was kept in an incubator at a temperature of 37° C.

Lot 2 was kept in an incubator at a temperature of 21° C.

The remaining lots were all kept at 21° C., but were kept under different conditions.

Lot 3 was 10 c. c. of semen plus 2 c. c. of urine from the horse.

Lot 4 was 20 c. c. of semen kept in a rubber bag.

Lot 5 was 20 c. c. of semen kept in a gelatin breeding capsule.

Time.	Lot 1. Per cent.	Lot 2. Per cent.	Lot 3. Per cent.	Lot 4. Per cent.	Lot 5. Per cent.
9:30 a. m.	100	100	100	100	100
10:30 a. m.	95	95	95	95	95
11:30 a. m.	75	95	75	90	90
1:00 p. m.	55	80	30	75	80
3:15 p. m.	1	70	20	20	75
5:30 p. m.	dead.	50	15	dead.	40
10:30 p. m.		10	0		dead.

Mounted specimens were made from the semen obtained from the services on November 8, 14 and 20 for the purpose of making measurements to note any decrease or variation in size from the nor-

mal or average size. Ten cells were measured in each preparation and the averages of the ten measurements gave the following results:
Average length of cells from service on Nov. 8 was 10-202 mm.
Average length of cells from service on Nov. 14 was 10-200 mm.
Average length of cells from service on Nov. 20 was 10-195 mm.

This shows only a very small difference in size of the cells from the last of the series of services and from those from the service of November 20, which are probably normal in respect to size, number, and vitality. Other measurements made and noted under a separate topic in this bulletin show about the same results.

Experiment No. 4. In this test it was decided to breed the horse twice a day for a number of days, collecting samples at intervals during the period of service. Also to have chemical analyses made of the semen at intervals during the test. The services were begun on December 14, 1908, and continued up to and including December 24. The horse was bred twice each day during this period with the exception of December 15, when no service was had.

The following table will show the general condition of vitality and number of sperm cells present on the dates when semen was collected for microscopic examination:

Table III

Date of Service 1908	Time Semen Lived at 18-25° C.	Time Semen Lived at 30-37° C.	No. of Sperm Cells, cu. mm.
Dec. 16	18° C. cells dead after 8 hours	35° C. cells dead after 6 hours	68,500
Dec. 18	18° C. cells dead after 7 hours	35° C. cells dead after 3.5 hours	23,500
Dec. 19	23° C. 5 per cent alive after 6 hours	37° C. 5 per cent alive after 4 hours	27,500
Dec. 21	18° C. 25 per cent alive after 3 hours	36° C. cells dead after 4 hours	36,500
Dec. 22	18° C. 15 per cent alive after 3 hours	37° C. cells dead after 1.5 hours	10,000
Dec. 24	20° C. 25 per cent alive after 2.25 hours	30° C. cells dead after 1.5 hours	23,000

The following summary shows the number of the service from which the material was secured for examination:

The service on December 16 as given in the table was from the third service.

The service on December 18 as given in the table was from the seventh service.

The service on December 19 as given in the table was from the tenth service.

The service on December 21 as given in the table was from the thirteenth service.

The service on December 22 as given in the table was from the sixteenth service.

The service on December 24 as given in th etable was from the twentieth service.

There were twenty services in this test, two services daily except on December 15. The samples recorded in the table were all from the first service for that date, with the exception of the samples collected on December 19 and 22, these being from the second service for those dates.

While no laboratory examination was made of the semen from the first service in this test it is safe to assume that the condition of the semen as to vitality and number of sperm cells would be about the same as has been found for this horse in previous experiments. For this data the reader is referred to the service of March 30 in Experiment 2, October 27 or November 20 under Experiment 3. The horse was not used for any breeding experiment after November 20 until the beginning of this test, December 14.

Briefly we may say that in this experiment the number of sperm cells dropped from 200,000 or more per cubic millimeter to 23,000 in the same quantity of semen in twenty services, and the vitality of the cells when kept at from 18° C. to 23° C. dropped from seven to nine hours at the beginning of the test to three to five hours at the last of the test. It is likely that the results obtained in these three experiments with this stallion are comparable with those that might be obtained from the best and most vigorous stallions, and certainly much better than would be obtained generally from horses of a sluggish temperament, an example of which is given in Table No. 1.

During the experiment samples of semen were sent to the chemist for analysis, especially for the determination of water and solids.

The following results of chemical analysis of the semen were reported by the Experiment Station chemists, C. Beatty and R. O. Baird:

Date 1908.	Service.	Water. Per cent.	Solids. Per cent.	Nitrogen. Per cent.	Protein. Per cent.
Dec. 16	Third	98.018	1.982	7.476	46.925
Dec. 18	Seventh	98.433	1.567	5.810	36.313
Dec. 19	Ninth	98.533	1.467	5.852	36.575
Dec. 21	Thirteenth	97.907	2.093	6.034	37.713
Dec. 24	Twentieth	98.143	1.857	6.720	42.000

The nitrogen and protein percentages are percentages of the total solids. The samples taken on December 19 and 24 were slightly reddish when dry. Some sediment was noticed in the beaker after these samples had stood for a short time. The material for analysis was taken so as to exclude the sediment. The analyses seem to indicate alternating conditions of the semen. This alternating condition was also noticed with reference to vitality and number of sperm cells present. This condition is illustrated by the tables under all of the experiments (four experiments) to determine the effect of continuous service. In the tables giving the summaries of these experiments it will be noticed that the number of sperm cells and their vitality do not decrease regularly on succeeding days, but fluctuate from day to day, but always with a tendency to a reduction in number and vitality when a number of services are considered.

SIZE OF REPRODUCTIVE CELLS

In practically all cases in the animal kingdom the egg cell or ovum is much larger than the sperm cell. This difference in size is largely due to the accumulation of food in the egg which serves as nourishment for the developing embryo. In some cases, as in the egg or ovum of the mammals, this food material is present in very small amounts, while in the eggs of birds it forms practically the entire bulk of the egg. The sperm cell is without any stored food material, and for this reason is much smaller than the egg cell. The sperm cell is also the actively moving cell while the egg, or ovum, is the passive or immobile cell.

The general shape of the sperm cell is the same for many of the higher forms of animal life. Figure 2 illustrates the shape of the cells as obtained from the horse. Those from cattle and hogs have

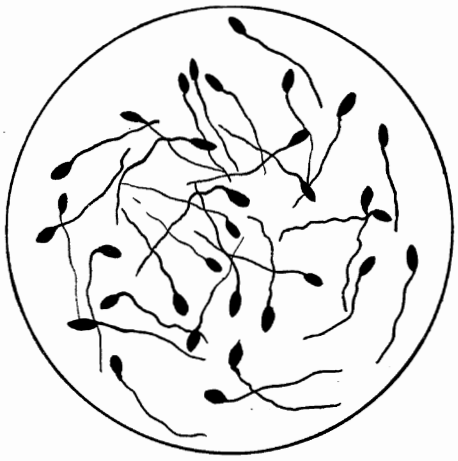


FIGURE II
Sperm cells from the horse magnified about 300 diameters.

the same shape. In connection with certain phases of this work numerous measurements of sperm cells were made. These measurements were made from the sperm cells from horses, cattle and hogs; also measurements were made from cells from the same individual under different conditions. The cells will vary slightly in size when collected from any service of the male, but as a rule they are very uniform in size and appearance, not only in any given service, but when collected under varying conditions. The sperm cells of the horse seem to decrease in size after frequent services. However, only one series of measurements were made with this particular object in view. The following measurements are selected at random from the many determinations that were made in connection with the work. Each series of measurements given will be for five cells and for any individual cell the column of figures will be read down, there being three measurements made for each cell. The figures given are fractions of a millimeter:

Length of head of sperm cell	1-110	1-119	1-110	1-110	1-110
Breadth of head of sperm cell	1-277	1-277	1-238	1-238	1-277
Total length of sperm	1-19	1-19	1-19	1-18	1 19

The above measurements were made from sperm cells obtained from a mature boar that had been for a short time on a diet of corn meal and cotton seed meal, equal parts. The following series of measurements are taken from semen obtained from a mature hog on a feed of corn meal and bran:

Length of head of sperm cell	1-110	1-110	1-122	1-110	1-110
Breadth of head of sperm cell	1-236	1-275	1-275	1-275	1-275
Total length of sperm	1-19	1-19	1-18	1-18	1-18

A large number of measurements were made of the sperm cells from different hogs and from the same hog under very different conditions. The variation in the size of the cells is very small, as most of the measurements run approximately as those given above for the two individuals tested.

The sperm cells from the horse are smaller than those from the hog. Under laboratory conditions the sperm cells from the hog usually retain their vitality much longer than those from the horse, and the suggestion naturally presents itself as to whether there is any relation in a given group of animals between the size of the cell and its vitality. The difference in size being mainly in the head or nucleus of the sperm.

Measurements of Sperm Cells From Stallion

Length of head of cell	1-165	1-183	1-183	1-165	1-165
Breadth of head of cell	1-366	1-366	1-366	1-330	1-336
Total length of cell	1-18	1-18	1-18	1-17	1-18

A second group of measurements are given from the same horse, the semen being taken from the eighth consecutive daily service:

Length of head of sperm cell	1-200	1-183	1-183	1-200	1-183
Breadth of head of sperm cell	1-366	1-366	1-366	1-366	1-366
Total length of cell	1-19	1-18	1-18	1-18	1-18

Continuous service, while it reduces the vitality and number of sperm cells, appears to have but little effect upon their size.

The sperm cells from the bull appear to more nearly approach those of the hog in size and activity as observed under the microscope. So far but little semen has been obtained from the bull, not sufficient to make definite comparisons with semen from other animals as to length of time the cells would retain their vitality under laboratory conditions.

Semen From Bull, Obtained August 28, 1907

Length of head of sperm cell	1-122	1-113	1-122	1-127	1-122
Breadth of head of sperm cell	1-120	1-236	1-236	1-236	1-236
Total length of cell	1-14	1-16	1-15	1-15	1-15

As stated above, the ovum or egg cell is much larger than the sperm and varies in size in the different species of animals, depending largely upon the amount of nutritive material stored in the cell. A number of measurements are given as taken from the different material examined.

Reproductive Glands

The cells concerned in fertilization are products of special glands of the body. These cells are necessary for the propagation of the species, and in order for conception or fertilization to take place both cells, the sperm and the ovum, must be physiologically active.

The ovaries are the glands of the female supplying the ovum or egg cell. This cell is much larger than the sperm cell, is globular in shape, and probably will live for a much greater length of time under either natural or artificial conditions than will the semen cells, although we have not determined this by observation in the case of the mammals. The ovum or egg is developed in a special portion of the ovary, the Graffian follicle, until matured. After maturity the follicle ruptures and the ovum with a considerable quantity of fluid escapes and is carried by the oviduct into the horn of the uterus. The general appearance of the Graffian follicle is shown in Figure 4, which shows

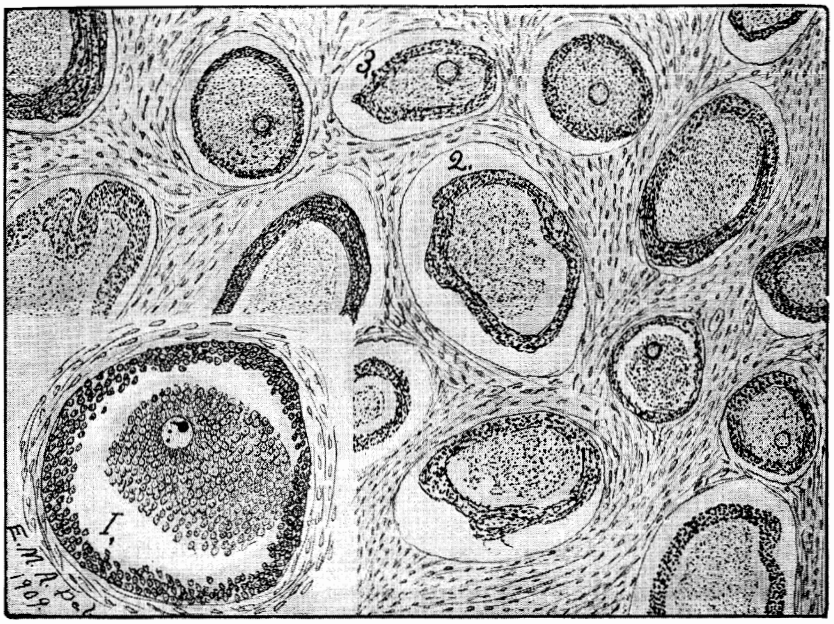


FIGURE III

Section of ovary from sow showing the ovum or egg in the follicles. 1. A matured egg cell. 2. Follicle in which the egg is not shown. 3. An immature follicle.

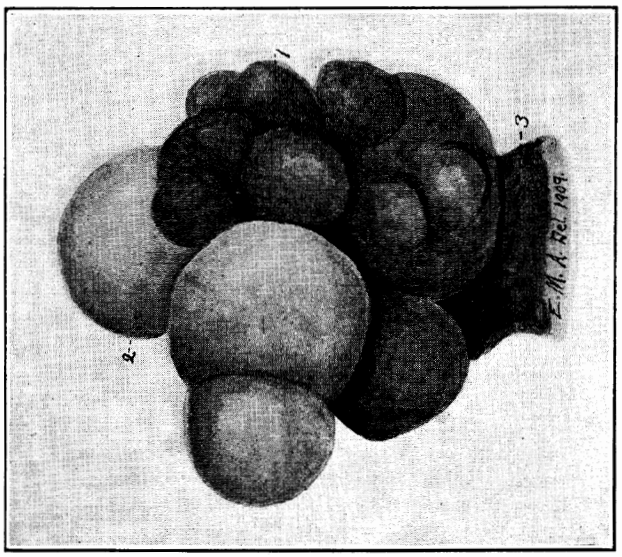


FIGURE IV

Figure of ovary showing four well developed Graffian follicles. 1. Immature follicles. 2. Fully developed follicles. 3. Attachment of broad ligament.

four well developed follicles. The period of heat in the female generally coincides with the rupture of these follicles.

There is no difficulty in determining whether the ovum has escaped from the ovary or not. The appearance of the ovary when the follicles are fully developed is very marked as compared with the ovary after the rupture of the follicles. When the follicle ruptures there is a slight hemorrhage and a collapse of the follicle. The point where the ovum escapes from the ovary is marked by a hemorrhagic spot that is very distinct for several days after ovulation.

The testes are the glands of the male which give rise to the sperm cells. These cells are much smaller than the ovum, but are not necessarily more susceptible to any influences that are unfavorable to their vitality than are the ova. The sperm cells are formed in the tubules of the testes, are passed out through these tubules to the vas deferens into the receptacle from which they are expelled during copulation. In their progress certain fluids are mixed with the cells which gives greater volume to the tesicular product. The sperm cells of many of the higher animals have much the same general form and vary but little as to size.

Figure 5 is a drawing made fram a section of the testes of the hog. The forming sperm cells which are shown by small black streaks can be seen among the cells lining the tubules of the gland.

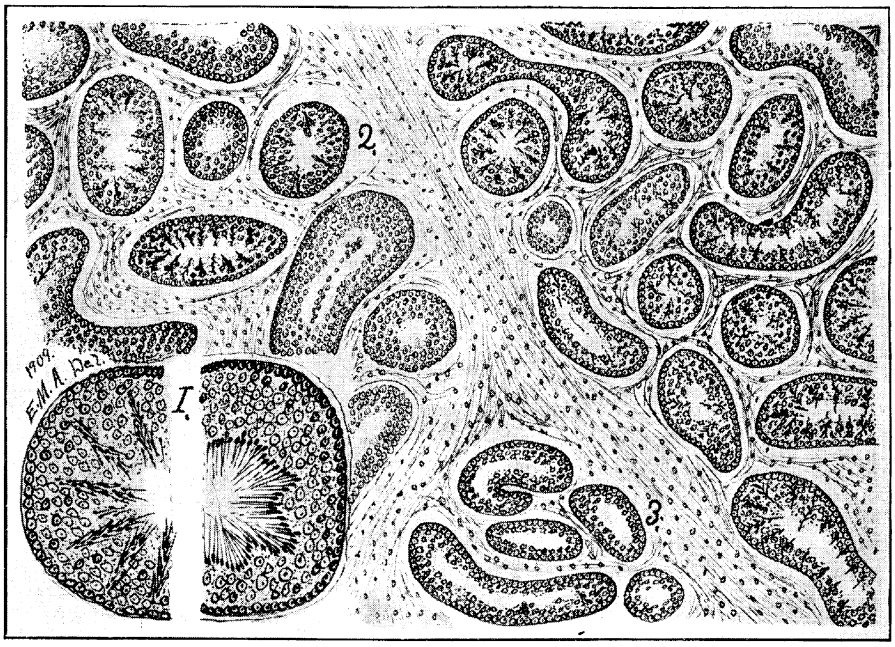


FIGURE V

Section showing the structure of the testicle from hog. 1. Tubule showing two stages in the passage of sperm cells into tubules. 2. Connective tissue of gland. 3. Tubules in which sperm cells are not forming.

Under a high power of the microscope some of the tubules have the

appearance shown in the larger figure in the lower lefthand corner of the drawing. This larger view of the tubule shows two stages in the passage of the sperm cells into the lumen of the gland. That portion of the figure to the left shows them in bands extending toward the lumen of the gland, while that portion to the right shows the spermatozoa practically free in the tubule. The formation of the sperm cells is not going on actively in all portions of the tubules of the testes at the same time. Some of the tubules in the testes, as shown in the illustration, give no evidence of sperm formation, and serial sections made from tissue of this character show that there are portions of the tubules of considerable length showing no activity in this direction. It is not probable that any portion of the gland in a healthy animal remains inactive for any great length of time, but it is true that all portions of the gland are not equally active at all times.

The glands from the mule, both male and female, were obtained for examination for the purpose of comparing their structure with corresponding glands from animals that will breed. Figure 6 shows the character of the tissue of the ovary. There was a dense connective tissue structure radiating apparently from the point of attachment of the main structure at the hilas of the gland. This con-

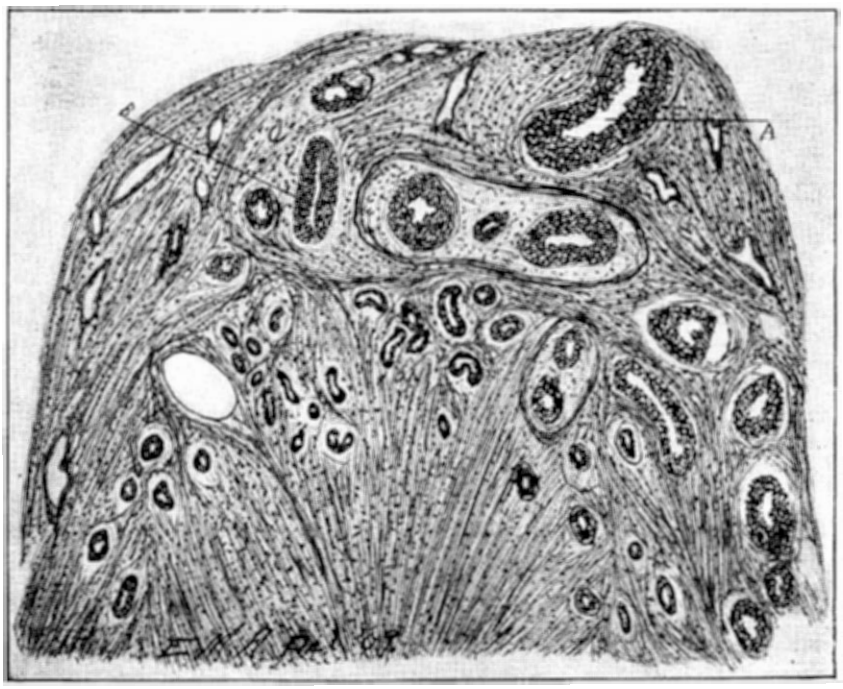


FIGURE VI

Section of ovary from mule. A and B, bloodvessels. No Graffian follicles in the gland.

nective tissue center is surrounded by a looser layer of tissue containing a large number of blood vessels. No structure resembling follicles could be found in any portion of the gland.

In the spring of 1908 two two-year-old mules were obtained in order to secure material from them for microscopic examination. They were allowed to serve several times and small quantities of material resembling semen from the stallion was secured. This material was entirely devoid of sperm cells. Probably a dozen tests were made from these mules, always with the same results, no indication of sperm cells. Figure 7 is from a section of the testicle obtained from one of these mules. By comparing this figure with Figure 5 the close resemblance of the gland of the hybrid and of the fertile male will be noticed. The tubules, connective tissue and other anatomical structures of the testicle of the mule are very much the same as will be found in the horse or hog. No trace of sperm cells or any indication of their formation could be seen in any of the sections made from these glands.

Effect of Various Conditions, as High Temperature, Sunlight, Chemicals, Etc., on the Vitality of the Semen

A number of experiments were conducted where the temperature for some of the semen was run as high as 52° C. In all of the tests run the high temperatures were quickly fatal to the cells. The following table is selected as giving a fair example of the results ob-

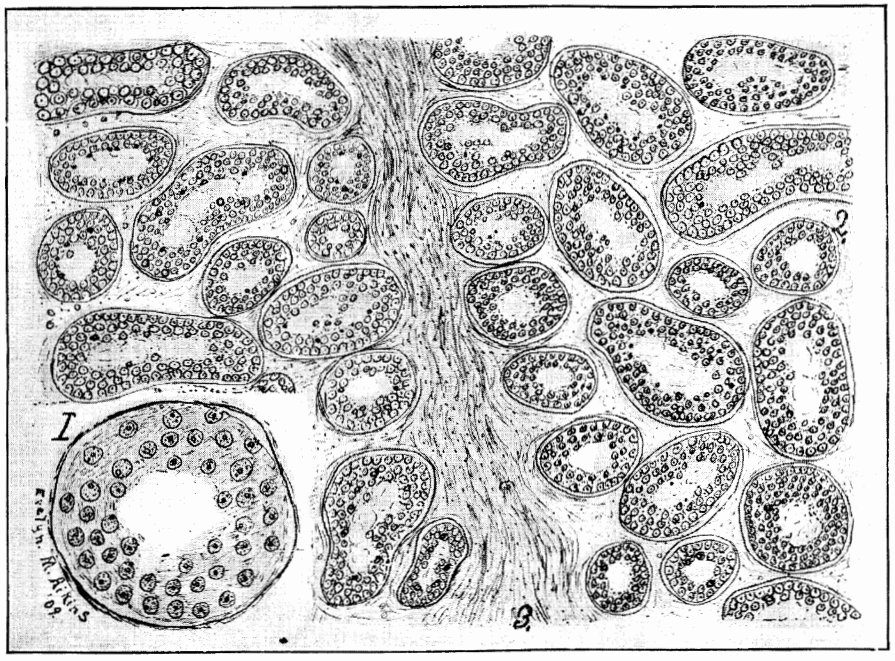


FIGURE VII

Section of testicle from a two-year-old mule. 1. An enlarged drawing of tubule designated by Figure 2 in drawing. 3. Connective tissue of gland.

tained by using a number of different temperatures on semen obtained from the same service. The stallion was bred at 9:30 a. m. on August 29 and a considerable amount of semen collected in a breeding bag. This was immediately divided into a number of lots and placed in sterile tubes and exposed to the following temperatures:

Time.	30° C. per cent alive.	32° C. per cent alive.	35° C. per cent alive.	38° C. per cent alive.	40° C. per cent alive.	43° C. per cent alive.	46° C. per cent alive.
9:40 a. m.	100	100	100	100	100	100	100
9:50 a. m.	100	95	90	90	75	15	10
10:00 a. m.	95	85	80	75	50	dead	dead
10:10 a. m.	90	75	60	40	25		
10:20 a. m.	80	60	40	30	20		
10:30 a. m.	80	60	35	25	20		
10:40 a. m.	80	55	35	20	10		
11:00 a. m.	75	40	20	5	2		
11:30 a. m.	70	30	15	5	dead		
11:45 a. m.	70	20	10	3			
12:15 p. m.	60	10	5	dead			
1:00 p. m.	55	dead	dead				
2:00 p. m.	20						
3:00 p. m.	10						
5:00 p. m.	dead						

A count of this lot gave 353,000 sperm cells per cubic millimeter. The observation at 9:40, or five minutes after the semen was distributed, showed the sperm cells very active at the lower temperature, but this short exposure had made a noticeable difference in the movement of the cells in the samples kept at the higher temperature, as the higher the temperature the slower the movement, and the sample at 46° showed scarcely any movement at all, although none of the cells appeared dead.

On May 9, 1908, a quantity of semen was obtained from the grade stallion. Portions of this semen were kept under such a variety of conditions that the results are given, especially as the minimum temperature is less than was used for most of the other experiments:

Time.	Per cent. 16° C.	Per cent. 20° C.	Per cent. 24° C.	Per cent. 34° C.	Per cent. 42° C.
10:00 a. m.	85	85	90	75	40
11:30 a. m.	75	70	75	50	dead
1:00 p. m.	60	55	45	20	
2:30 p. m.	50	40	20	dead	
4:30 p. m.	20	10	7		

This semen was collected at 9 a. m. and carried immediately to the laboratory, where it was divided into lots of 15 c. c. each

and placed at the various temperatures as above indicated at 9:30. Samples of semen have been cooled as low as 12° C. for a short time and the only immediate effect noticed was the slow movement of the cells as compared with portions of the same semen when kept at or near body temperature.

In another test where the semen was kept in a number of vessels made from different materials, certain results were obtained that are of interest. The grade stallion was bred on May 13, and 125 cubic centimeters of semen was obtained in the breeding bag. A part of this semen was placed in a bag of parchment paper and placed in the vagina. This was done at 8:30 a. m., and the remainder of the semen was carried to the laboratory for tests. Bags were made of gelatin (breeding capsules), rubber, parchment, and membrane from pig's bladder. The rubber and pig's bladder were thoroughly washed before placing the semen in them. The rubber was a portion of a breeding bag that had been used in the experiments. All of the semen, with the exception of the lot in the ice box, was kept at 23° C. and in the diffused light of the laboratory.

Time.	Ice box. 18° C.	Glass vessel.	Gelatin capsule.	Parchment bag.	Rubber bag.	Pig's bladder.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
9:00 a. m.	100	95	95	95	60	90
10:00 a. m.	96	90	90	85	40	75
11:00 a. m.	85	85	80	70	15	30
12:00 m.	75	75	75	60	1	5
1:30 p. m.	50	65	70	50	dead	dead
3:00 p. m.	45	50	60	40		
5:00 p. m.	35	30	45	30		
7:30 p. m.	30	15	35	15		
9:30 p. m.	25	5	10	5		

Other experiments were made along the same lines as indicated above and the results from rubber and membrane from pig's bladder always indicated that the material was harmful to the sperm cells. Semen allowed to remain in the breeding bag at body temperature rapidly loses its vitality, until within a comparatively short time all of the cells are dead.

At 2 p. m. the parchment bag that was inserted into the vagina was secured and a careful examination showed that all of the cells were dead, while that portion of the semen kept in a parchment bag in the laboratory at 23° C. showed approximately 50 per cent alive. As stated above, there were 125 cubic centimeters of this semen. The count showed that there were 197,000 sperm cells in each cubic millimeter, or more than twenty-four billion of cells in the semen secured at one service. Experiments were conducted where the temperature

was as high as 51.5° C. In all of the tests run the general effect of high temperature was the same, that is, it soon destroyed the vitality of the cells.

As has been noted before, the vitality of the semen may be materially altered by mixing various materials with it. Even the addition of water will alter the vitality, as shown by the following experiment. In addition to the samples that had water added, samples were carried through in the breeding bag and in glass vessels. All samples were kept at even temperature, 21° C.

The semen was collected from the grade stallion on April 26 at 8 a. m. The semen was examined as soon as it reached the laboratory and practically all of the cells were alive. The semen was divided into five lots: Lot 1 remained in the breeding bag; Lot 2 was in a clean glass vessel. The remaining Lots, 1, 2 and 3, had 10 per cent, 5 per cent, and 3 per cent of water added, respectively:

Time.	Lot 1.	Lot 2.	Lot 3.	Lot 4.	Lot 5.
	Per cent.	Per cent.	Per cent.	Per cent.	Per cent.
8:00 a. m.	100	100	100	100	100
9:00 a. m.	30	75	10	20	20
10:00 a. m.	5	60	5	15	15
11:00 a. m.	2	45	1	10	8
12:00 m.	dead	75	dead	2	1
1:00 p. m.		15		dead	dead

SUMMARY

First: The vitality of the reproductive cells of the hog is only a few hours. In most cases the ovum appears to lose its power of being fertilized within 48 hours, and the sperm cell does not appear to possess much if any greater vitality.

Second: The number and vitality of the sperm cells from the stallion decrease rapidly where one or two services are given daily.

Third: The ovum (in hogs) is not liberated from the ovary until the last part of the period of heat.

Fourth: The sperm cells are very sensitive to high temperatures and chemicals. Apparently low temperatures have less effect on their vitality than any other conditions tested.

ERRATA

Page 18, sixteen lines from bottom, for page 14 read page 16.

Page 22, second line from top, for pages 16-21, read pages 18-21.

On title page, for Veterinary, read Veterinary Science and Bacteriology.