FACTORS INFLUENCING THE FERTILITY OF NATURALLY AND ARTIFICIALLY MATED SWINE

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ABSTRACT

FACTORS INFLUENCING THE FERTILITY OF
NATURALLY AND ARTIFICIALLY MATED SWINE.

by Anthony Borton

The problems of boar semen collection, extension, handling and preservation require particular attention since many of the techniques developed for artificial insemination of other species are not applicable. Additional difficulties are encountered with the insemination of the sow and accurate estrus detection. Therefore, the artificial insemination of swine has developed slowly in the United States and conception rates have generally been low. This study was concerned with fertility of artificially inseminated swine and with boar semen preservation and handling techniques. Comparisons were made in the fertility of naturally and artificially mated swine.

A series of laboratory experiments on the preservation of boar semen did not indicate any improved storage methods. Although satisfactory spermatozoa motility was maintained, the pregnancy rate of boar semen stored 5 to 10 days was 9.1%. The fertility of stored boar semen was not closely related to spermatozoa motility.

The methods of semen collection and extension and artificial insemination techniques were described. The standard insemination dose was 5×10^9 spermatozoa diluted to a 50 ml. volume with an egg yolk-sodium bicarbonate-glucose extender. The diluted semen was deposited in the female tract with a plastic dairy inseminating catheter connected by a piece of tubing to a 50 ml. plastic syringe.

Anthony Borton

A swine artificial insemination field trial comprised of 654 matings was conducted at Southern Michigan State Prison. Complete breeding and farrowing information was available on 438 predominately gilt inseminations. A farrowing rate of 32.0% was obtained with an average litter size of 7.0 pigs. A significantly higher ($P < 0.01$) farrowing rate was obtained with two services during estrus (44.5%) than with one service (21.3%). There was also a highly significant ($P < 0.01$) difference in the farrowing rates obtained from the three boars used in the study. The least square estimate of the difference between the high and the low boars was 19%. Three inseminators also obtained significantly ($P < 0.05$) different conception rates. The storage age of semen, up to 30 hours, did not significantly influence swine fertility. The progressive and total motility of boar spermatozoa was not closely associated with subsequent farrowing rates. The backflow of semen from the female tract following service did not indicate an improper insemination technique since those sows exhibiting backflow had a significantly ($P < 0.01$) higher farrowing rate than those that had no backflow. None of the factors studied had a significant influence on litter size.

Artificial insemination was compared with natural breeding in 374 matings on the Michigan State University swine farm. A farrowing rate of 64.5% and an average litter size of 10.0 pigs obtained on 254 natural matings was compared to a 50.0% farrowing rate and a 9.3 average litter size on 120 artificial inseminations. These differences were not signi-

ficant when analyzed by the method of least squares. The boar was the only factor examined that had a highly significant ($P < 0.01$) influence on farrowing rate in this study. The differences in farrowing rates between sows or gilts and one or two services during estrus were not significant. Since the farrowing rate increased with twice serviced females in the A.I. group but decreased with twice bred females in the natural group, there was a significant interaction ($P < 0.05$) between type of mating and number of services. A similar interaction ($P < 0.05$) was also observed for litter size. Differences in litter size by breeding seasons were highly significant ($P < 0.01$). The litter size was significantly ($P < 0.01$) larger (1.96 pigs) for sows than for gilts.

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by

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I. INTRODUCTION

The enthusiastic acceptance of artificial insemination revolutionized dairy cattle breeding in this country. From a modest beginning in 1938, the artificial breeding industry has grown to include over 40% of the dairy cattle and 5% of the beef cattle annually. The economic and safety advantages, as well as the possibilities for health and genetic improvement have contributed to this popularity. Artificial insemination techniques have been adapted for routine use with poultry, goats and dogs and to a limited extent for horses, sheep and swine.

The expansion of artificial insemination of swine has been slow since most of the cattle techniques are not applicable to swine artificial breeding. In addition, in the United States research and field trials have been plagued by discouraging conception rates. Accurate heat detection and reduced fertility following semen storage have presented additional problems. Nevertheless, producer interest has remained high as it offers distinct advantages to present management practices. Swine artificial insemination is suited to programs of disease free pigs, feeder pig production and carcass improvement.

In 1959 Michigan State University initiated its investigations in swine artificial insemination (A.I.). Initial semen collections were made on the University swine farm using an artificial vagina and a teaser sow. Insemination was accomplished with plastic squeeze bottles and dairy inseminating catheters. In the fall a boar stud was established in conjunction with the Endocrine Research Unit, a cooperative laboratory

between the Departments of Animal Husbandry and Physiology.

The boar stud was organized by N. L. First and since its inception has been under the direction of J. E. Nellor, Director of the Endocrine Research Unit. Initially 5 boars were housed and trained for semen collection in a converted draft horse barn. In the fall of 1959 an experimental field trial on the fertility of artificially inseminated estrous-controlled gilts was conducted under the direction of N. L. First, J. A. Hoefer and J. E. Nellor. An orally active progesterone was fed for 15 days and the gilts artificially inseminated when they exhibited standing estrus following the last hormone feeding. A farrowing rate of 54% was obtained on the 39 gilts synchronized for estrus and serviced artificially.

Additional studies were conducted to examine A.I. techniques in swine on the University Farm. Within a year the boar stud was expanded to include 8 boars, and over 100 University sows and gilts had been inseminated. E. C. Miller, A. Borton and J. E. Nellor conducted a series of studies on estrous synchronization and artificial insemination at the Endocrine Research Unit which, in the fall of 1960, culminated in a field trial. One hundred and fifty two gilts, on 11 cooperating central Michigan farms, were put on treatment and inseminated during the controlled estrus by artificial insemination. No consistent response was observed, as results varied from herd to herd.

The artificial insemination program continued to expand and on March 10, 1962 new facilities for the Endocrine Research Unit, including a seperate Artificial Insemination Research Boar Stud, were completed.

The present facility, managed by A. Jaworski, was the first one constructed specifically as a research center for swine A.I., and afforded the possibility of expanded research programs and field trials.

To date at Michigan State University nearly 40 boars have been trained and semen has been collected in excess of 2,000 times. More than 1,500 females have been inseminated either in the campus herd or in the field. The work presented in this report was initiated to: (1) examine methods of handling and preserving boar semen and (2) to investigate factors influencing fertility of artificially inseminated swine. In order to attain these objectives a number of storage techniques were examined, and field trials were conducted at the Michigan State University swine farm, the Southern Michigan State Prison farm and the Ionia Reformatory farm.

II. REVIEW OF LITERATURE

The Semen of the Boar

Collection procedure

The development of artificial insemination in any species requires a simple and efficient method of obtaining semen from the male. Many methods of semen collection from a boar have been attempted. Walton (1933) described early methods where semen was withdrawn from the vagina of a mated sow by suction or with a sponge. Coronel and Masankay-Arenas (1954) collected semen as it dripped from the external genitals of a sow during mating. Recently Mihailov (1963) described a new method of obtaining boar semen by inserting a removable tampon into the uterine cervix of the sow and collecting the ejaculate as it runs out of the vagina. The problem of contamination in any of these methods is obvious.

Hammond (1957) noted that, whereas in the ram and bull coitus is quick and the ejaculation mechanism is temperature sensitive, coitus in the boar is a prolonged affair and ejaculation is stimulated by pressure. Numerous artificial vaginas have been designed for collecting semen from the boar. Ito et al. (1948a) observed that the average boar ejaculation lasted 6 minutes 36 seconds (range 2 min. to 23 min. 35 sec.) and collection equipment must make use of pressure stimulation throughout this period. Various types of artificial vaginas have been described (Glover, 1955; Polge, 1956b; Turkheimer et al., 1958; Aamdal, 1960) all of which make use of internal pressure to initiate ejaculation. Many are fitted with rubber bulbs to control pressure levels and provide

pulsating pressure, while others make use of hand pressure or a spiral wire into which the boar's penis locks.

Hancock and Hovell (1959) reported the collection of semen by hand using a rubber glove. The "artificial vagina" is made by forming an opening in the hand with the encircling thumb and index finger. Ejaculation is evoked by applying pressure to the spiral end of the boar's penis. Although this method is attributed to Niwa (1958), a modification of this principle was used earlier by McKenzie et al. (1938) where a rubber tube was placed around the penis instead of the rubber glove. Dziuk (1963) and other workers in this country have been using the "glove" method for several years.

Collection by electrical stimulation was reported by Vera Cruz (1959), and Arhiporec (1961) developed an apparatus which fractionates the semen at collection. Some mention should also be made of Beseth's (1962) findings which indicate a sensitivity of boar spermatozoa to certain artificial vagina rubber liners.

Boars can be collected off a "teaser" sow as described by McKenzie et al. (1938) or they can be readily trained to mount a "dummy" (Rodolfo, 1934b; Hancock, 1959c). Descriptions and illustrations of various types of "dummies" are cited by Glover (1955), Niwa et al. (1959a), Rowson (1959), and Aamdal (1960).

The question of boar nutrition and management under a program at a boar stud has been investigated by Hanson (1951), Nikulina and Ermakova (1962) and Stevermer et al. (1961). In general, they observed that exercise, direct sunlight and pasture were not essential for the

production of fertile semen. When boars were raised in confinement there was no appreciable difference in libido or semen characteristics. In addition, widely varying planes of nutrition can be tolerated by boars without detrimental effects on spermatozoa. Dziuk (1962) related that enforced exercise over an obstacle course was required of boars at studs in France.

Ejaculate characteristics of the boar.

Volume, spermatozoa concentration and fractions.

The boar's ejaculate is unique among the domestic animals. The composition of the semen and its manner of ejaculation were thoroughly described by McKenzie et al. (1938). More recent reports (Ito et al., 1948a; Niwa, 1958; Hancock, 1959c; Self, 1959; Gerrits et al., 1962) generally agree with this earlier work. The boar ejaculate, characterized by a large volume (average 250 ml.), contains four distinct fractions: presperm, sperm rich, post sperm and plug. The plug averages about 20% of the total ejaculate but can range from 5% to nearly 50%.

Spermatozoa concentration is in the neighborhood of 150 to 250 x 10^6 per milliliter in the entire ejaculate but averages 600 x 10^6 in the sperm rich fraction. Self (1959) reported that by collecting only the sperm rich fraction, 60% of the total ejaculate volume could be discarded with only a loss of 20% of the sperm numbers. Niwa (1958) observed that most of the sperm cells are ejaculated in the first half of a collection, 82% appearing in the first two minutes. The sperm rich fraction was "ejaculated undulatingly", appearing as waves. The majority of the ejaculates contained only one wave, but instances of four waves and irregular waves were also reported.

Frequency of ejaculation

Wallace (1949) noted that when normal boars were regularly collected over an extended period of time there were considerable variations in both semen volume and spermatozoa concentration. Similar variations have been reported by McKenzie et al. (1938), Ito et al. (1948c) and Niwa (1958). They also observed that frequency of ejaculation was an important factor affecting not only semen volume and concentration but also sperm morphology and duration of motility.

A boar should not be used more than once every two weeks according to Rodolfo (1934a). Niwa (1958) stated that, with a collection interval of three days, you can expect about 70-80% of the normal spermatozoa output and an interval of 5 to 6 days avoids any effect on the semen volume and sperm concentration. He also stated that a vigorous boar can be used daily but twice daily collection is over use of a boar. Pitkjanen (1962) observed that a boar avoided the dummy after two days on a twice daily collection schedule. On a once daily collection the volume remained constant but sperm concentration fell after a week. At the end of five weeks of daily collection libido was diminished. A once a week collection schedule resulted in no variation in sperm number of semen volume. Gerrits et al. (1962) noticed similar results on a daily collection schedule with the exception that there was a significant reduction in semen volume, and occasionally the ejaculate was devoid of spermatozoa. A 48 hour collection interval resulted in the greatest total sperm production. By examining reserves of epididymal spermatozoa in the boar, Singh (1962) estimated that the average daily production of spermatozoa was 13.9×10^9. On the basis of these findings he concluded that a

3 to 4 day interval between collections was necessary for optimal semen concentrations.

Radford (1961c) observed that variations in boar ejaculates depended far more on the type of artificial vagina used than on the frequency of collection. Although the variation in volume with collection interval was not significant in boars over two years old, there was a significant ($P < 0.01$) increase in the sperm concentration as collection interval increased. He recommended a four day interval for immature boars and a two to three times a week collection for mature boars. On this schedule he estimated that the average mature boar could produce semen annually for 1,000 to 1,500 inseminations and the immature boar semen for 550 to 600 inseminations.

McKenzie et al. (1938) reported an increase in abnormal spermatozoa with increased frequency of collection and considerable variations between boars. Gerrits et al. (1962) confirmed this finding. Niwa (1958) and Gerrits et al. (1962) estimated the average percentage of abnormal sperm in healthy boars to be between 5 and 10%. McKenzie et al. (1938), Niwa (1958), Hancock (1957b) and Hancock (1959c) described the types of abnormal sperm found in boar semen. The highest proportion of the abnormal spermatozoa were either immature with protoplasmic drops or coiled-tail spermatozoa.

McKenzie et al. (1938) reported that the duration of sperm motility increased as the interval between collections increased. The average duration of motility of samples collected at 12 hour intervals from 5 normal boars was 2.5 days while the duration of motility increased to

8.5 days in samples from a 72 hour collection interval. Pitkjanen (1962) also noted that spermatozoa from samples collected once or twice weekly survived longer than in samples collected more frequently.

Age of boar.

A major factor affecting the composition of the ejaculate is the age of the boar. Turkheimer et al. (1958), Bane (1959), Niwa et al. (1959b) and Radford (1961c) observed that in young boars the semen was of a poorer quality than in mature boars. Initial ejaculates were often contaminated with urine, low in volume and sperm concentration, and contained a large number of immature or abnormal spermatozoa. However, with increasing age, a successive improvement in semen quality occurred. Turkheimer et al. (1958) obtained first ejaculations from young boars at 120 to 133 days of age and by 34 to 46 weeks semen output per ejaculate had reached a high level. Niwa et al. (1959b) also noted the appearance of spermatozoa first at four months and after six months spermatogenesis was described as vigorous.

Season of the year.

The season of the year also influences the composition of the ejaculate. Okauchi and Hirakata (1962) reported that semen volume was lowest during the seasons of maximum and minimum environmental temperature, although this did not have a significant influence on the numbers of spermatozoa. Stevermer et al. (1961) agreed that volume increased during the moderate fall months but they also noted that total spermatozoa numbers were highest in the summer. (June through August). During the summer a decrease in viability and the percentage of live

spermatozoa was observed by Okauchi and Hirakata (1962), Mizuho et al. (1963b) and Corteel (1963). At the French boar stud in Poitou Corteel (1963) noted that the rejection rate of ejaculates due to dead or a high percentage of abnormal spermatozoa was five times greater from August to November than it was for the remainder of the year.

Chemical composition of boar semen.

The first major investigation into the chemical properties of boar semen was undertaken by McKenzie et al. (1938). They analyzed boar semen for chlorides, glucose, urea, creatinine, solids and total organic nitrogen. It wasn't until Mann's (1954) studies on the biochemistry of semen that complete semen analyses were reported. His book presents an excellent review of the chemical composition of the boar's ejaculate. Additional information can be found in the works of Glover and Mann (1954), Glover (1955), Niwa (1958), Polge (1959), Self (1959),Bialy and Self (1959), Aalbers et al. (1961), and Hartree (1962). They substantiate Mann's report that the boar seminal plasma has an unusually low content of fructose and sorbitol and a remarkably high content of citric acid, ergothioneine and inositol as compared to other animals.

Recent emphasis on the biochemistry of boar semen revolves around the work of Graham (1963) in Minnesota. Analysis of the carbohydrate components of semen indicated that fructose, glucose and sorbitol are metabolized, while mannitol and glycerol serve as intermediates. The amino acids of boar semen were also isolated and identified. Glutamic and aspartic acid concentrations were as high as 60 mg. %. The total

nitrogen content of boar semen ranged from 400 to 800 mg. % of which 14 to 28% was non-protein nitrogen. The Minnesota researchers are also studying the phospholipid content of boar semen. Graham and Mann, as well as others, emphasize the marked variability in composition of boar semen. Extreme variation exists between boars as well as a large and significant variation between ejaculates from the same boar. It is interesting that many of the factors which affect ejaculate volume and concentration are also reported to affect its chemical composition.

Boar Semen Storage

Temperature

The boar ejaculate has already been described as differing from that of the bull and ram by virture of its large volume and comparitively low sperm concentration. In addition, researchers have found that boar spermatozoa are unique in their behavior *in vitro*. Niwa *et al.* (1959a) reported that 15° to 20° C was the optimal temperature for maintaining motility in the whole ejaculate. The sperm survival at these relatively high temperatures was remarkably longer than that observed with bull and ram spermatozoa. Boar semen storage at the "usual" temperatures (around 5° C) resulted in spermatozoa death. By contrast, the sperm rich fraction of boar semen is similar to other species in that sperm survival at high temperatures is relatively short and motility can be maintained at temperatures of 3° to 10° C. Polge (1959) suggested that some factors in the boar seminal plasma were responsible for the high temperature survival, and that bull and ram spermatozoa stored in boar seminal fluids had increased survival at 20° C.

Aalbers et al. (1961) noted several other unique features of the boar ejaculate. Whereas bull and ram spermatozoa maintain their motility equally well under aerobic and anaerobic conditions, boar spermatozoa are comparatively ineffective anaerobes. Under aerobic conditions the R.Q. of the boar spermatozoa equals that of the ram and bull and boar spermatozoa were able to oxidize lactic acid (the main product of anaerobic fructolysis) as well as other substrates. However, it was reported that boar spermatozoa were incapable of oxidizing sorbitol. Aalbers et al. (1961) also noted that oxygen uptake of the boar ejaculate was not entirely by the spermatozoa, but was in part by the seminal plasma itself. In fact, even with a declining motility the oxygen consumption increased upon storage in vitro.

Anabiosis

Another peculiarity of boar spermatozoa concerns their behavior during storage, wherein they quickly lose their motility and settle out. This property of boar semen has been referred to by the early Russian and Japanese workers (Ito et al.,1948c) as anabiosis. Sperm cells that have undergone anabiosis can be re-activated by warming to 37° C and shaking in the presence of air. As demonstrated by Polge (1956b) and Niwa (1958) reactivation may require 2 or 3 hours following a period of storage. This peculiarity of boar semen must be considered in any study in which motility estimates are made of stored samples.

Mizuho et al. (1960) observed a close relationship between metabolism and anabiosis. When boar semen was stored at low temperatures anabiosis occurred earlier than when semen was stored at higher

temperatures. In an attempt to control this sedimentation or anabiosis, Wettke et al. (1962b) examined the influence of adding gelatin to various storage diluents. In all diluters studied gelatin was effective in reducing or preventing sedimentation, but sperm motility was also reduced compared to gelatin free controls. Mizuho et al. (1960) also mentioned that anabiosis was prevented and metabolism maintained, even at low temperatures, by shaking. This may have a practical implication for transported semen, since the shaking in transit could cause a loss in energy and viability through maintenance of high metabolic levels. Prokopcev (1963) also noted that agitation was harmful to stored semen. Samples that had been agitated had an increased pH.

Diluters and extenders.

With these behavior peculiarities in mind, research was undertaken to formulate diluters and storage procedures specifically for boar spermatozoa. The ideal diluter should serve to preserve spermatozoa, extend the volume, prevent temperature shock and provide a buffer system without any loss in fertility. The early Russian diluters, as described by Walton (1933) and Rodin and Lipatov (1936), contained glucose and small amounts of sodium sulphate or sodium potassium tartrate and peptone. Ito et al. (1948c) preserved undiluted semen at 20° C for 5 days but vigorous motility was retained for only about two days. They also noted that Kreb's phosphate, Ringer's solution and physiological saline did not harm the sperm cell and could be used to increase volume. The advent of sperm diluents containing egg yolk by Lardy and Phillips (1939), or milk and milk products (Thacker and Almquist, 1951) resulted in con-

siderable investigation into their use for boar spermatozoa. While Polge (1959) indicated that boar spermatozoa would not survive at 5° C in the typical yolk-citrate medium used for bulls, both milk and egg yolk have become important components of recent boar semen extenders. Jakobsen and Mann (1960), while working on biochemical methods of appraising semen quality, noted that the action of a milk diluent was not so much to provide an additional nutrient substrate as it was to provide a protective or life preserving effect. They ascribed similiar actions for egg yolk and other colloidal substances. These diluter components acted not to increase the initial rate of metabolism but rather to maintain it over a period of time. On the other hand, Mizuho et al. (1963a) reported that both egg yolk and milk not only preserved good sperm motility but also caused an acceleration of respiration and glycolysis.

A large scale study of over 200 extenders was conducted at Cornell (Young et al.,1957) where various combinations of sodium citrate, sodium phosphate, sodium bicarbonate, glucose, glycine, glycerol, inositol and egg yolk were examined. They observed the best survival in a diluter of 20% egg yolk, .33% citrate, .33% glycine and 1% glucose stored in sealed vials at 15° C. The addition of .75% sodium bicarbonate and 1.25% inositol also gave satisfactory results. They reported 20% egg yolk superior to 50%. Dzuik (1958a) examined over 400 semen samples in 26 diluters. From a motility basis he observed the most satisfactory diluters contained glucose or glycine and egg yolk or milk. Optimum motility was attained when semen was stored at 7° C in a diluter of 3 gm. glucose, 0.15 gm. sodium bicarbonate, 30 ml. (30%) egg yolk and 70 ml.

distilled water plus penicillin and streptomycin. Niwa (1958), Hess et al. (1960a) and Feredean et al. (1962) also obtained best results with egg yolk diluters.

Irwin (1959) compared glycine and citrate in egg yolk and milk at different temperatures and obtained the most satisfactory results with a combination of egg yolk and milk at 5° C. Pásztor and Tóth (1962) compared six milk diluents; heated skimmilk with 2% egg yolk gave the best sperm survival. Paredis (1959) also reported that skimmilk powder with some egg yolk made a more satisfactory diluter than egg yolk alone.

Subin (1962) tested diluents containing egg yolk, citrate, glucose, bicarbonate, or milk stored at three temperatures; the greatest motility was maintained at 15° to 20° C when the extender contained bicarbonate, citrate or milk. No advantage was obtained by adding egg yolk or glucose to milk. All diluents were unsatisfactory at storage temperatures of 0° C. Whole milk or skimmilk as extenders have been popular with other workers. Although Dzuik (1958a) obtained optimum motility with a yolk extender he also received good motility results with a skimmilk-glucose-distilled water extender. Niwa et al. (1959e) reported satisfactory results with a milk powder diluent. Self (1959), Bialy and Self (1959) and Stratman (1961), maintained sperm motility for the longest period at 9° C storage with a heated homogenized whole milk extender. Several heated skimmilk-glycine-egg yolk diluters were also examined and found to be superior to the egg yolk-glucose-bicarbonate diluter.

Mizuho et al. (1963a) depressed sperm respiration and glycolysis

with the addition of sugars to milk and egg yolk extenders. Pásztor and Tóth (1962) indicated that the addition of glucose did not increase spermatozoal survival. Radford (1961d) augmented semen with fructose and increased survival, although it was not sufficiently improved to be considered a good storage technique. Numerous other ingredients have been utilized. For example, Arhiporec (1962) benefited motility with small amounts of ascorbic or salicylic acid while Prokopcev (1963) utilized boric acid.

Sodium citrate is a common ingredient of bull diluents. Noll (1949), Young et al. (1957), Hammond (1957), Dzuik (1958a), Gotink (1959) and Polge (1959) however, reported that citrate appeared to be toxic to boar spermatozoa. Aamdal and Hogset (1957) and Bane (1959), in contrast, obtained satisfactory conception rates with an egg yolk-sodium citrate diluter. This anomaly was explained in part by Niwa et al. (1959d), who indicated that sodium citrate was harmful to semen unless it was mixed with egg yolk at a rate not to exceed 4 to 1. Another common ingredient of bull extenders, for freezing, is glycerol but Young et al. (1957), Polge (1959) and Graham (1963) observed that glycerol decreased boar sperm motility.

The inclusion of antibacterial agents in semen extenders is a common practice. Yoshida et al. (1951) examined various agents for bacterial control in boar semen stored at 20° C. The addition of streptomycin, sodium sulphamerazine, sodium sulphadiazine, homosulphamine or boric acid increased the duration of boar spermatozoa survival. The sulpha compounds were more effective when combined than when used alone.

Mizuho et al. (1963a) noted that streptomycin and penicillin in combination were suitable, but chlortetracycline adversely affected spermatozoa at concentrations compatible with antibacterial efficiency. Coronel and Masankay-Arenas (1954) tested the effect of antibiotics in eleven different diluters and concluded that variations in viability of boar spermatozoa were as much due to quality of individual samples as to the different types and proportions of antibiotics. However, antibacterial agents are widely used in swine semen diluters and have been demonstrated by Polge and Rowson (1956) to improve fertility. The most popular antibiotic combination at present is streptomycin and penicillin.

Spermatozoa survival in storage was the highest when the seminal fluids were removed (Stratman 1961). Removal of the accessory fluids by centrifugation was discussed by Bialy and Self (1959) and Self (1959). Centrifuging significantly lowered the motility of stored spermatozoa compared to non-centrifuged controls after two days storage. Dott and Walton (1960), working with ram semen, commented that resuspension of the sperm cells was difficult after centrifuging semen. Manipulation of spermatozoa resulted in increased agglutination or clumping of motile spermatozoa. This phenomenon is a common problem and its occurrance has been recorded by Bialy and Self (1959) and Aalbers et al. (1961).

Although Noll (1949) reported no difference in sperm survival under anaerobic or aerobic conditions, Young et al. (1957) obtained a significantly better survival of semen stored in sealed ampullas as opposed to corked tubes. Even though sperm activity was reduced in a CO_2 atmosphere no increase in survival was obtained by Polge (1959) with

storage in either a CO_2 or nitrogen atmosphere at 20° C. Arhipovec (1961), Ben Joseph and DuMesnil du Buisson (1962), DuMesnil du Buisson and Dauzier (1959) and Foley (1962) have experimented with the effect of CO_2 saturated diluters or CO_2 atmosphere on boar sperm survival, the French workers reporting excellent results.

Investigations into the effect of osmotic pressure on the preservation of boar spermatozoa have been conducted by Stevermer et al. (1962, 1964). Using a skimmilk diluter, osmotic pressure was controlled by adding mesoinositol or D-fructose. Motility was highest between freezing point depressions of -.420 and -.548° C. Fertility, based on only limited breedings, was significantly ($P < .05$) greater at a freezing point depression of -.536° C compared to -.420° C and -.480° C. They concluded that osmotic pressure may be a more important factor in maintaining motility and fertility than is generally recognized.

Numerous ranges of temperatures have been investigated for the storage of boar sperm. It appears that 5 to 12° C is most satisfactory for fractionated ejaculates and 15 to 20° C for whole ejaculates. The rate of cooling of the sperm cell is of equal importance. The phenomenon of "temperature shock" long recognized to occur in the semen of other species has not been critically studied in boar spermatozoa. Since the volume of the ejaculate is large it is not as obviously susceptible to environmental temperature variations. Lasley and Bogart (1944) examined the influence of cold shock on boar spermatozoa and observed that epididymal spermatozoa had a higher resistance than did ejaculated spermatozoa. However, investigation revealed that the secretions of the

accessory glands did not in themselves greatly influence the resistance of the boar spermatozoa. Further, they observed that the fluids of the epididymis did not increase the resistance of ejaculated spermatozoa but an egg yolk-phosphate buffer did provide temperature shock protection for both epididymal and ejaculated spermatozoa.

Diluted boar semen suffered less damage from slow cooling than from quick cooling (Willems, 1959). Slow cooled spermatozoa maintained a 60% motility at 4 days while quick cooled spermatozoa had a rapid loss in motility after 24 hours. Feredean et al. (1962) reported that the optimum cooling time for boar spermatozoa to refrigerated temperatures was 10 to 12 hours. Dzuik (1958) suggested a rate of less than 5° C per hour. Mizuho et al. (1963a) observed temperature shock to be more marked in rapidly cooled samples.

The role of semen dilution and its interrelationship with spermatozoa resistance to cold shock has been the subject of conflicting reports. Choong and Wales (1962) reported that diluted suspensions of bull spermatozoa were more susceptible to cold shock than more concentrated samples. However, Lasley and Bogart (1944) indicated that the degree of dilution had no influence upon the resistance of boar spermatozoa to cold shock.

A deleterious effect of dilution on the boar sperm cell was suggested by Kvasnitsky (1959). Ito et al. (1948c) and Niwa (1958) recommended that dilution not exceed 1:6 and preferably less for semen extended with physiological saline at the time of insemination. Rodin and Lipatov (1936) noted that a 4 fold extension gave satisfactory results.

Boar spermatozoa did not survive as well in higher dilutions of 1:20 as they did in lower dilutions of 1:2 or 1:5 (Polge 1959). On the other hand, Dziuk (1958) indicated that 1:1 dilutions had lower motility on storage than 1:2, 1:4 or 1:8 dilutions. Mizuho et al. (1963a) reported that with rapid addition of diluter the depression of motility and metabolism were proportional to the extent of dilution. The degree of dilution shock varied according to the temperature at which dilution took place and little damage was done to sperm cells in a 1:10 dilution if the dilution was made gradually. Holt (1959) observed that a dilution of more than 1:10 could be used without a loss of fertility.

In summary, a number of diluters, temperatures and techniques for the storage of boar spermatozoa have been examined but no completely satisfactory method has been developed. Diluting fluids containing either milk or egg yolk have been satisfactory for the maintenance of motility for about 7 to 10 days at refrigerator temperatures. The longest duration of motility noted in this review was 19 days at 12° C. (Irwin, 1959). It was observed by Aalbers et al. (1961) that a large proportion of boar spermatozoa could be regarded as motile during storage, but only a very small portion could be regarded as progressively motile. Critical motility estimates are further hampered by agglutination of spermatozoa, anabiosis and the accompanying necessary reactivation process. El Sheikh and Casida (1954) suggested that with rabbit spermatozoa motility in vitro and fertility may not be similar physiological phenomena. Hammond (1957) noted that many researchers have observed that fertility was lost even though motility was retained.

Self (1959) observed that the percentage of motile spermatozoa was not significantly correlated with the percentage of fertilized ova obtained with the same sample. While there was a highly significant association ($r = 0.89$, $P < 0.01$) between percent fertilized ova and the type of motility in fresh semen this relationship did not exist in stored semen. The data of Stevermer et al. (1964) indicated that motility of stored spermatozoa was not a reliable indicator of fertility. It is apparent that significant contributions to boar semen storage methods must be accompanied by fertility information.

Freezing boar spermatozoa.

To date attempts at storage of boar semen by freezing have not been successful. Many (Roy, 1955; Hess et al., 1960b; Hoffman, 1959; Bane, 1959; Niwa et al., 1962; Dukelow and Graham, 1962; Settergren, 1958; Polge, 1959) have obtained some motile spermatozoa after thawing frozen semen but only two investigations have reported any conceptions resulting from the use of frozen boar semen. Hess et al. (1957) artificially inseminated 25 gilts with boar semen which had been stored at -95° C for 1 to 19 days. The gilts were slaughtered 20 to 60 days following A.I. Seven of the gilts had an average of 9.4 embryos. Hoffman (1959) inseminated 11 sows with semen frozen at -79° C and one sow farrowed a litter of nine pigs. This was the only record found of a litter produced with frozen boar semen.

Although there have been few successes with frozen boar semen some important contributions have been made. The Nördiska Veterinarmötet Report (1958) along with Settergren (1958) established that boar semen

froze better in small rather than large containers. The largest volume they successfully froze was 15 ml. in long narrow tubes, but Hess et al. (1960b) reportedly utilized 50 ml. heat resistant bottles. Polge (1959) and Hoffman (1959) observed sperm survival best when the seminal plasma was removed before freezing; however, Niwa et al. (1962) determined that the seminal secretions did not have adverse effects.

Clycerol has been an essential ingredient in bull and ram freezing diluters, but the glycerol levels necessary for best cell recovery from freezing caused a decrease in motility of the boar spermatozoa (Young et al. 1957, Polge 1959 and Graham 1963). Even though it appears that glycerol is more toxic to boar spermatozoa than to bull or ram spermatozoa, it was an ingredient in all the freezing attempts where satisfactory motility was observed following thawing. Recent work by Niwa et al. (1962), Dukelow and Graham (1962) and Graham (1963) indicated that short equilibration times of 4 to 6 hours with 5 to 7% levels of glycerol were the most satisfactory for maximum recovery of spermatozoa motility.

Dukelow and Graham (1962) reported the most critical temperature for freezing boar semen was between -8° C and -38° C. Freezing rates of 1° to 3° C per minute were recommended. Niwa et al. (1962) proposed a differential freezing rate of $\frac{1}{2}$° C per minute to -10° C, 5° C per minute to -30° C and 5° to 10° C per minute down to -79° C. The maximum motility following freezing was 60% reported by Niwa et al. (1962).

Skimmilk, whole milk, egg yolk, glucose, glycine and sow's milk

have been tried as components of frozen boar semen extenders. The problem of freezing boar semen with successful preservation of its fertilizing ability, however has not been resolved.

Physiology of Breeding the Sow

Natural mating

The natural mating of a boar and a sow is a prolonged event. The boar will spend considerable time pushing and nudging the sow prior to mounting. If the sow is in estrus she will stand quietly and accept the overtures of the boar. Although Milovanov and Sokolovskaya (1946) and Burger (1952) believed the boar's penis penetrated the cervix and ejaculation occurred into the uterus, more recent work by Smith and Nalbandov (1958) indicated that the penis penetrates only midway into the vaginal portion of the cervix. In contradiction to Burger they reported the cervix of the sow did not relax but rather constricted at estrus. Utilizing a system of radiographs they also observed that the penis only penetrates the cervix to the depth of a few centimeters, where the annular rings seem to grip it, bringing about ejaculation. These findings concur with earlier reports by McKenzie et al. (1938), Anderson (1945), Hammond (1957) and Polge (1956a) that semen was deposited in the vaginal portion of the cervix. The large volume of the ejaculate evidently forces semen into the uterus and the gel plug serves to reduce its flowback. McKenzie et al. (1938), Anderson (1945) and Burger (1952) observed that although the plug will effectively seal the cervix following a natural mating it is not indespensible for successful conception.

Artificial insemination procedures.

Catheters

The insemination catheters developed for artificial insemination of swine have been designed with the anatomy of the cervix in mind. Most commonly used in this country are the plastic dairy insemination catheters, which many workers modify by corkscrewing the end to fit into the annular rings of the cervix. Almquist (1959) summarized the inseminating equipment used in other countries, including catheters made of glass, rubber, ebonite and plastic. Aamdal and Hogset (1957) developed an insemination tube that had an inflatable balloon near the tip that was designed to seal the cervix and thereby force semen into the uterus. Melrose and O'Hagan (1962) compared this Norwegian catheter to other varieties and in general found no significant difference in conception rates due to type of catheter. However, they did find the Norwegian catheter superior to the Japanese hard rubber catheter described by Niwa et al. (1959c).

Site of semen deposition in artificial insemination.

The actual extent to which the insemination catheter penetrates is the subject of some controversy, although it is generally agreed that the site of deposition depends on the age of the pig and the type catheter used. Smith and Nalbandov (1958) examined the size of the cervical opening and expressed doubt if even the smallest of their probes entered the uterine portion of the cervix, much less the uterus itself. Melrose and O'Hagan (1962) examined the passage of catheters at different stages of the estrous cycle and found the deepest penetration occurred

when animals were not in estrus. Japanese researchers (Ito et al., 1948b) place the site of insemination as the second prominence of the cervix uteri. This it appears that artificial insemination at estrus deposits the semen in approximately the same place as in natural mating, and penetration of the uterus by the pipette does not occur.

Holt (1959) and Hancock (1959a) however, reported fertility differences based on intrauterine and intracervical inseminations. Fertility was higher when semen was reportedly deposited in the uterus than when placed in the cervical canal (Hancock,1959a); conception was not obtained with vaginal inseminations. On the other hand, Holt (1959) observed no difference in conception of intrauterine as compared to intracervical insemination but convencing proof of the extent of penetration of the catheter was not presented. Paredis (1961a) found no relation between the depth of insemination and conception rate.

Semen injectors.

Because of the relatively large volumes involved, several methods of placing the semen into the female tract have been developed. Almquist (1959) describes the use of syringes, gravity flow bottles, infusion pumps and plastic squeeze bottles as "injectors". Gusev (1962) describes a disposable plastic bag, the content of which is emptied into the insemination tube by squeeze pressure. Holt (1959) observed that the conception rate was slightly higher with insemination by gravity flow instead of pressure, but the highest conception occurred when a combination of the two methods was employed. DeMesnil du Buisson (1961) reported inseminating 10,000 sows in the field by means of flow

by gravity. The time required for 250 ml. was 4 to 7 minutes, the speed being dependent on uterine contractions on the part of the sow.

Stimulation at mating.

Hammond (1957) and Polge and Rowson (1956) suggested that considerable stimulation of the female cervix occurs at natural mating and may be involved in a subsequent oxytocin release, which in turn could influence uterine muscle contractions. Artificial insemination therefore may be deficient in stimulating one of the physiological mechanisms involved in sperm transport. It would appear also that gilts and sows should be handled quietly prior and during insemination since adrenalin release has been shown to counteract the action of oxytocin (Polge and Rowson, 1956).

Pitkjanen (1955) suggested that the act of mating may influence the time and number of eggs shed. Examination of sows slaughtered following estrus indicated that 88% of those mated had shed eggs compared with 44% for those not mated. When sows were mated to a vasectomized boar 28.5% more ovulated than with the unmated control.

Sperm transport.

It is generally accepted that the horns of the uterus are filled with semen soon after mating. Spermatozoa reach the utero-tubular junction within 10 minutes (Mann et al., 1956) but the passage into the oviducts may be somewhat slower. Pikjanen and Subin (1961) detected no spermatozoa in the oviducts $1\frac{1}{2}$ hours after insemination although some spermatozoa had reached the mid-portion 2 hours after A.I. Ito et al. (1944) determined that semen reached the Fallopian tubes $\frac{1}{2}$ hour after

mating. They also observed, as did DuMesnil du Buisson and Dauzier (1955b), that the rate of transport of spermatozoa was affected by the stage of estrus, being more rapid toward ovulation.

The large volume of a boar's ejaculate may be necessary to fill the greater length of the sow's uterine horns, (Hammond, 1957). In addition the sow possesses less uterine fluid at the time of estrus than does the cow and ewe. DuMesnil du Buisson and Dauzier (1955a), Mann et al. (1956) and Lovell (1958) noticed that the large volume of the boar ejaculate was quickly absorbed, the bulk disappearing within 5 to 8 hours. Seminal secretions and a large percentage of spermatozoa disappeared from the tract during this time. Spermatozoa disappearance was least and survival greatest at the utero-tubular junction. The rate of disappearance did not appear affected by phase of estrus. Ito et al. (1944) ascertained that spermatozoa viability in the sow's tract could last 42½ hours and maintain fertilizing ability for 25 to 30 hours. Pitkjanen and Subin (1961) estimated that 80% of the spermatozoa in the oviducts were motile after 6 hours and some progressively motile spermatozoa were present in the horns after 48 hours. McKenzie et al. (1935) recovered viable spermatozoa from the Fallopian tubes 5 hours after mating.

Optimum time to service.

Since the viability of the ova and spermatozoa in the female tract is limited, it is important that service, either natural or artificial, are in proper relationship to the time of ovulation. Hancock and Hovell (1962) observed that fertilization failure was the greatest single cause of low conception when inseminations were made too early

or late in the estrous period. This was interpreted as a failure of the spermatozoa to reach the ova rather than a loss of fertilizing ability, since spermatozoa were not found in the zona pellucida of the ova. Mating late during estrus also increased the frequency of polyspermic fertilization in swine (Hancock, 1959b) and might represent a source of embryonic mortality in A.I. swine.

Ito et al. (1944) studied normal estrus in swine and reported the optimal time of service was 10 to 25.5 hours after the onset of estrus. A Wisconsin field trial involving several hundred sows (Self, 1961) indicated that sows inseminated within 12 hours after the onset of estrus had a significantly ($P < 0.01$) higher farrowing rate than sows serviced over 24 hours after the start of estrus. This relationship did not apply to gilts where no significant difference in conception rate due to time of insemination was evident. Bane (1959) and Madden (1959a) also obtained highest fertility with sows inseminated in the first 24 hours of estrus. On the other hand, Rodin and Lipatov (1936), Stratman (1961) and Hancock and Hovell (1962) obtained a higher fertility by breeding on the second day of estrus in sows. Madden (1959b) could not determine a pattern in fertility of gilts bred 1 to 60 hours after the onset of estrus. Radford (1961b) reported a higher farrowing rate for gilts bred on the third day of estrus as compared to the second day, irrespective of whether they stood to be served.

Madden (1959b) determined the first post weaning estrus was more fertile than subsequent ones, but with a limited number of sows Self (1961) reported the conception rate highest when sows were bred on

the second day of the second post lactation estrus.

Sow behavior at breeding.

Behavior at the time of service may influence conception. Radford (1961b) obtained a higher farrowing rate from sows that stood firm when serviced compared to those that moved. Madden (1959b) acquired a 7% difference in farrowing rates in favor of sows that stood during service. No relationship was noted between swelling or reddening of the vulva and conception. Holt (1959) also utilized the swelling of the vulva as a guide to stage of estrus but found it unreliable. A 63.8% farrowing rate was obtained when sows were "very quiet" at breeding but only a 34.1% rate when they were "agitated" (DuMesnil du Buisson, 1961). Self (1962) also reported a significantly higher rate for sows and gilts in standing estrus.

Artificial Insemination of Swine

Artificial insemination has not developed as rapidly with swine as it has with some other species, primarily because of some of the problems already cited. The first relatively large scale use of artificial insemination was with horses around the turn of the century. Professor Elie Ivanov of the Russian State Stud, the father of modern A.I., was named director of the Russian Central Experimental Breeding Station in 1919 and he instigated research on preservation of semen and insemination techniques in all species. An excellent history of these early years of artificial insemination was presented by Walton (1933). The Russians' first published report on swine artificial breeding was presented by Milovanov (1934). McKenzie, Lasley and Phillips (1939) were the first

to report successful artificial insemination of swine in this country. From this beginning swine A.I. is now under investigation in many countries and in commercial operation in several.

Methods of calculating fertility.

Conception rates for swine cannot be accurately calculated on a non-return basis. Swine are not usually maintained under intensive surveilance by the farmer and consequently estrus detection is not very accurate. An artificial insemination trial of over 1,000 sows was reported by Holt (1956) where the non-return rate was calculated as 71%. At farrowing, however, only 14% had litters. Polge and Rowson (1956) decided that 30-60 day non-return figures bore little relation to farrowing, figures being 65% for the former and 24% for the latter. Instances of gilts and sows failing to show estrus after breeding and then proving to be non pregnant are common (Hancock and Hovell, 1961; Melrose and O'Hagan, 1959; Polge and Rowson, 1956). The major reason for infertility of sows has been attributed to embryonic death (Warnick et al., 1949). The conception rate is always lower if calculated from fetuses present at 25 days than if calculated from sow's with cleaved ova at 3 days (Self, 1959; Stratman and Self, 1960). Considerable fertility research has been reported on the basis of 3 day slaughter data. Self (1959) and Dziuk (1960), however, emphasize the errors inherent in this system where fragmented ova may be mistaken for fertilized ova. First et al. (1963) indicated that 3 day slaughter data may still have validity if the spontaneous cleavage rate is determined for the population under investigation.

Although several methods have been used to calculate swine fertility, the most desirable is farrowing rate based on first service, along with average litter size (Almquist, 1959; Rowson, 1962). A report on swine artificial insemination in the European countries by Beattie et al. (1961) emphasized that farrowing rate may be misleading as a criterion of A.I. success if some of the sows were rejected by the inseminator at the time of service for not showing sufficient indications of estrus. To illustrate, they calculate that a 70% farrowing rate with a 15% rejection rate means that only 58.5% of the females presented to the inseminator became pregnant.

Several workers have compared the conception rates from first services with repeat breedings. Holt (1959) detected no difference in farrowing rates of first inseminated compared to reinseminated sows. Similarly, Madden (1959a) experienced no difference between first inseminations and repeat inseminations in the first 1,000 females serviced in a field study; however, later results indicated a slight but nonsignificant increase in favor of the first insemination. Self (1961) and Jacobsen (1959) observed a slight but nonsignificant increased farrowing rate in first serviced sows over reserviced sows. Whereas Self noted no difference in farrowing rates of first serviced and rebred gilts, Jacobsen (1959) observed that the reserviced gilts had a significantly ($P < 0.05$) higher farrowing rate.

Natural mating compared to artificial insemination.

Several workers (Stratman, 1961; Stratman and Self, 1962; Subin, 1962; Niwa, 1958) have reported no appreciable difference in conception

rates or litter sizes between sows bred naturally and those mated artificially. Others (Lovell, 1958; Paredis, 1961) observed that conception rates on artificially mated sows generally ran 10 to 12% under naturally bred sows. Hancock (1957a) ascertained that 91% of the ova from 15 naturally bred sows were cleaved whereas in four groups of sows serviced artificially the percent of cleaved ova ranged from 21.7% to 47.8%. An 86% farrowing rate with a 10.9 average litter size was reported by Madden (1959a) when 915 sows were mated naturally but when the same sows were serviced artificially during the next breeding season only 45% farrowed with an average litter size of 10.4. Paredis (1961) detected slightly higher litter sizes for natural mating than for A.I. (i.e. 0.6 pigs), but this difference was not statistically significant. Niwa (1958) observed that litter size as a result of A.I. does not differ markedly from that of natural mating. Paredis (1962) used the same 4 boars to breed 365 pigs, naturally and artificially, and obtained a 48% conception rate on the artificially inseminated females and a 34% rate on those naturally bred, these conception rates were below the local district averages.

Factors affecting fertility in artificially inseminated swine.

Diluters and extenders.

The components of swine semen extenders and their ability to maintain motility has already been reviewed but some workers have compared the fertility obtained with different diluents. Jokinen (1961) compared a sodium citrate-glucose-egg yolk extender with one of powdered milk-egg yolk and obtained no difference in conception rate between

diluters. An experiment comparing skimmilk-egg yolk, sodium citrate-egg yolk and glucose-egg yolk (Paredis, 1959) indicated little difference in farrowing rate between the three diluters. There was a slight improvement in favor of the milk-egg yolk diluter, however. Using the split ejaculate technique a slight but nonsignificant advantage in favor of homogenized milk extender with 10% fat as compared to the egg yolk-citrate extender was obtained by Jakobsen (1959), and similar results comparing the same diluters were reported by Hansen et al. (1960). With a 0.2% sodium bicarbonate-glucose-egg yolk extender Gotink (1959) obtained conception rates of over 75% in gilts. He found this buffer gave better results than skimmilk, glycine or glucose buffers.

Corteel (1963) examined the effect of CO_2 on farrowing rate using a split ejaculate technique. This resulted in a 39.05% farrowing rate with a 8.35 pig average with the IVT extender plus CO_2 gas and a 51.07% farrowing rate with a 9.51 pig average with the IVT alone. Subsequently a modified IVT without CO_2 gave a farrowing rate of 56.6% on 1373 first inseminations. Finally, Polge and Rowson (1956) showed that the addition of antibiotics resulted in a definite improvement in fertility and litter size of both 6 hour and 24 hour old semen that was diluted with a 2% glycine-egg yolk extender.

Motility.

The ineffectiveness of motility as a critical means of evaluating semen is recognized, but several workers have related semen motility to fertility. Selection of an ejaculate on the basis of initial motility is likely to increase the conception rate according to Paredis (1961),

who indicated that intensity of motility was related to pregnancy rate, particularly in the extreme cases. Paredis and Vandeplassche (1962) obtained a significantly ($P < 0.05$) higher conception rate when initial motility was greater than 75% than when it was below 75%. Higher incidences of pregnancy at 21 day slaughter were acheived by Hess et al. (1960a) when semen motility was in the 80-90% motility range prior to insemination. Niwa et al. (1959a) and Ito et al. (1948b) recommend that motility be at least 70% before an ejaculate is used for artificial insemination.

Stratman et al. (1958) detected no significant association between the percentage of motile spermatozoa and the percentage of fertilized ova at 3 days. When stored semen of 47% motility was compared to fresh semen of 95% motility, the conception rates were approximately the same. They concluded that motility was a satisfactory indicator of fertilizing capacity of fresh semen but was not of stored semen. On the contrary, the type of motility of stored semen was found to be closely related to fertility by Missouri workers. (Lasley and Day, 1960).

Volume and spermatozoa concentration.

The relationship between volume of semen inseminated and fertility achieved in swine may be extremely important. Hammond (1957) suggested that the large volume of the boar's ejaculate may be necessary to provide fluid to fill the greater length of the sow's uterine horn. Rodin and Lipatov (1936) inseminated 1, 5, 25, 50 and 100 ml. of fresh semen and recommended the larger volumes for the highest fertility. Wiggens et al. (1951) detected little difference in the fertility

(cleaved ova at 3 days) of sows inseminated with either 50 ml. or 250 ml. volumes. Self (1959) and Stratman (1961) reported no difference in conceptions with 50 ml. or 100 ml. of undiluted fresh semen. A 50 ml. volume produced a higher rate of embryo survival ($P < 0.01$) and conception rate ($P < 0.05$) than did 10 ml. or 20 ml. with varying spermatozoa concentrations (Stratman and Self, 1960). It was suggested that spermatozoa number did not affect litter size as much as did the volume inseminated.

Hancock and Hovell (1961) compared 20 and 120 ml. volumes and found no evidence that sperm transport was improved with the larger volume. This was estimated by the number of spermatozoa in the zona pellucida of recovered ova. Coronel (1953) reported that the semen volume was not related to litter size, but his data were limited and not analyzed statistically. He reported conceptions with as little as 10 to 12 ml. of semen. First et al. (1960), with 19 inseminations in each group, obtained a 42% farrowing rate using 35 ml. and 68% rate with 70 ml. Pitkjanen (1955) indicated that the fertilization rate was 54% greater with 200 ml. as compared to 100 ml. but unfortunately the number of breedings involved was not mentioned. Rothe (1960) found 100 ml. better than 50 ml. and Polge (1959) reported that conception results were lower with 25 ml. or 50 ml. than with 100 ml.

The role of semen volume in sperm transport is not thoroughly understood and the effect of sperm dilution in vivo has been studied. In this process a small concentrated amount of semen is first inseminated and this is followed with a large volume of diluter. Rodin and Lipatov

(1936) reported inferior results from attempts to dilute in vivo. Kvasnickii and Konjuhova (1960) obtained a conception rate of 91.6% when 20 ml. of undiluted semen was placed in the neck of the uterus and followed by 100 ml. of pure diluent and then 150 to 200 ml. of filtered air. Melrose and O'Hagan (1959) described a system where first 125 ml. of diluted semen was inseminated followed by 100 ml. of extender containing 25% freeze-dried gel. Although controls were not available for comparison their conception rate was not appreciably different from that of other workers. Hancock and Hovell (1961) inseminated one group of swine with 20 ml. of semen and a second group with 20 ml. of semen followed by 100 ml. of pure diluent. The first treatment resulted in a significantly ($P < 0.05$) higher fertilization rate and there was no evidence from the number of spermatozoa in the zona pellucida of recovered ova that sperm transport was enhanced by the larger volume.

The relationship between sperm concentration and insemination volume was examined by Polge (1959) who observed a 16% farrowing rate with a 7.7 pig average at a low concentration and volume (.5 to 1 billion spermatozoa in 25 ml.). At the highest concentration and volume studied (10 to 20 billion spermatozoa in 100 ml.) the farrowing rate was 74% with a 10.5 pig average. Paredis and Vandeplassche (1962) indicated that the number of spermatozoa retained in the female tract after insemination had a highly significant ($P < 0.01$) effect on conception rate. The increased conception that occurred was more pronounced in sows than in gilts. They also noted that litter size was significantly ($P < 0.05$) smaller when spermatozoa numbers were less than 2 billion. Self (1959)

and Stratman and Self (1960) reported no difference in conception rates or embryo survival between sperm concentrations of 2.5, 5 and 10 billion. In a later study Stratman and Self (1961) observed no significant difference in conception rates when 1.25 and 2.5 billion spermatozoa were inseminated. Dauzier and DuMesnil du Buisson (1959) reported that conception rates were reduced when less than 6 billion spermatozoa were inseminated. Paredis (1961) indicated that farrowing rate and litter size increased with increasing spermatozoa concentration up to 7 billion. On the other hand, Radford (1961b) observed that spermatozoa concentrations of 1 to 30 billion had no effect on farrowing rates or litter size. Plisko (1961) noted no difference in conception when from 0.5 to 10 billion spermatozoa were inseminated but he did find conception rates were lower when 0.3 billion spermatozoa were inseminated. Wiggens (1951), with small numbers of sows, realized no decrease in conception rate when as few as 0.2 billion spermatozoa were inseminated. Hancock and Hovell (1961) published results that indicate that in a 20 ml. volume 1 and 10 billion spermatozoa resulted in a significantly higher ($P < 0.01$) conception rate than 0.1 billion. It appears, therefore, that the optimum number of spermatozoa per insemination is somewhere between 1 and 7 billion.

The injection of oxytocin or its addition to boar semen had little influence on fertility of gilts inseminated with low spermatozoa numbers and reduced semen volume (Stratman et al., 1959; Self, 1959; Stratman, 1961). On the other hand, Milovanov and Sergeev (1961) and Sergeev (1963) indicated that litter size and conception rates were improved 8% to 20% over controls when oxytocin was added to the boar semen.

Semen backflow following insemination.

Those familiar with insemination of swine recognize that some backflow of semen may occur following service. Lasley and Day (1960) reported that difficulty was encountered in making the semen remain in the reproductive tract of the gilt following insemination. Ohio workers (Hess et al., 1960a) suggested that improper insemination technique could be detected by the backflow of diluted semen from the vagina. Polge and Rowson (1956) and Melrose and O'Hagan (1959) attempted to replace the natural plug and minimize runback by completing the artificial mating with the insemination of freeze-dried gel. They concluded that the presence of freeze-dried gel in a diluent does not appreciably affect conception rate. Polge (1956a) had previously suggested the use of a sterile cotton plug to control flowback but Dziuk (1958), Madden (1959), Radford (1961b) and Paredis (1961) reported that there was no relationship between the volume of semen lost during insemination and conception rate or litter size. A farrowing rate of 18% with no backflow was observed by Holt (1956), with some backflow it was 20% and with considerable backflow the rate was 27%. This difference diminished as overall conception improved but the farrowing rate for those with backflow remained higher than those with no backflow (Holt, 1959).

In vitro storage age of semen.

The effect of storage of semen on fertility is subject to some conflicting reports. Stratman et al. (1958) and Madden (1959b) reported no decrease in conception rate when semen was stored for 12 hours. Akatov et al. (1962) perceived no drop in either conception rate or

litter size with semen stored up to 24 hours. On the other hand, numerous workers (Paredis and Vandeplassche, 1962; Dauzier and DuMesnil du Buisson, 1958; Aamdal, 1959; Niwa, 1958; Feredean and Slavescu, 1961) observed a progressive, nonsignificant decline in conception when semen age increased from less than 2 hours to more than 24 hours. In no instance was the reduction more than 10%. However, Paredis (1962) observed a significant ($P < 0.05$) decrease in conception rate after 4 hours and a significant ($P < 0.05$) reduction in litter size after 2 hours storage. Litter size was the highest with fresh semen. Jacobsen (1959) obtained a significant decrease ($P < 0.01$) in farrowing percentage and litter size in sows and gilts when semen age increased from 2 up to 12 hours.

There is more conclusive evidence that a decrease in fertility occurs when semen is stored longer than 24 hours. Holt (1959) observed that the conception rate for semen stored over 24 hours was half of that for fresh semen. DuMesnil du Buisson (1961) reported on over 4,000 inseminations using semen less than 6 days old stored at 15° C under CO_2, and rediluted just before use. The overall conception rate was 51.3% and the conception rate for 33 sows bred with 4, 5 or 6 day semen was 25.4%. A progressive drop in conception rate was noted on each additional day of storage. Conception rates of 53.4 and 51.2%, respectively, were obtained with fresh and 24 hour old semen, the difference being nonsignificant. Litter size was reported to be normal for the breeds used.

Other researchers (Ito et al., 1948b; Hess et al., 1960a; Polge, 1959; Lasley and Day, 1960; Aamdal and Hogset, 1957) reported drastic decreases in farrowing rates and litter size following storage. Ito et al. (1948b)

also noted a slight decrease in the percentage of males at birth following *in vitro* storage of semen. Polge (1959) obtained a 72% conception rate with fresh diluted semen which dropped each succeeding day until after 4 days the conception rate was 13%. During the same period the litter size dropped from 10.7 to 7.3 pigs. The drop in fertility was greater than one would expect from motility estimations made prior to insemination. Saturation with CO_2 failed to improve the pregnancy rate obtained from stored semen.

The effect of boar semen storage on embryonic mortality in A.I. gilts was investigated by comparing cleaved eggs at 3 day slaughter to fetuses at 25 days (Dziuk and Henshaw, 1958; Dziuk, 1958b). Conception rates were reduced after 72 hours of storage and embryonic mortality was also higher than for semen stored either $\frac{1}{2}$ hour or 24 hours. No significant differences were found in conception rates (3 day slaughter) with semen stored either 6 hours or 54 hours (First *et al.*, 1963). However, significantly ($P < 0.01$) more ova were fertilized with 6 hour (61.0%) than with 54 hour semen (39.3%). The percentages of pregnancies and corpora lutea that were represented by 25 day embryos was greater for the 6 hour than for the 54 hour stored semen. They also observed that *in vitro* sperm age affected the number of spermatozoa in the zona pellucida of cleaved ova. Pitkjanen and Subin (1961) reported similar results when comparing fresh with semen stored 24 hours. Fewer spermatozoa of semen stored 72 hours were found in the oviducts, the percent sperm motility was less and the percentage of phagocytized spermatozoa was greater.

How long stored boar semen retains its fertilizing capacity is uncertain. Coronel and Masankay-Arenas (1954) reported a sow that farrowed 7 pigs with semen stored 5 days. DuMesnil du Buisson (1961) obtained fifteen pregnancies with semen stored 4, 5 and 6 days, while Niwa et al. (1959a) reported successful conceptions with 7 day old semen. Sows compared to gilts.

A 1961 report on artificial insemination of swine in European countries (Beattie et al., 1961) noted that in general the conception rate for gilts is lower than for sows. Jakobsen (1959) indicated a significantly ($P < 0.01$) higher farrowing percentage for 382 first service sows than for 260 first service gilts. With over 200 females in both groups Self (1961) also observed a significantly ($P < 0.025$) higher farrowing rate for sows than for gilts (46.1% to 35.4%). Other workers (Madden, 1959a; Paredis, 1962; Hess et al., 1960a; Larsson, 1960; Gotink, 1959; Holt, 1959; Bane, 1959; Feredean and Slauescu, 1961) reported conception rates to be 2 to 17% higher for sows than for gilts. Number of services in estrous period.

An early A.I. experiment at Missouri (McKenzie et al., 1939) achieved a 21% conception rate for gilts inseminated one time and a 53% rate for those inseminated two times during estrus. Weaver and Bogart (1943) cited J. F. Lasley's Missouri thesis work where gilts bred twice in the same estrus showed greater conception rates and farrowed more pigs than gilts bred once. Over a two year period Alexandrowicz (1955) also observed that double mating of sows increased the average litter size from 9.5 to 10.23 pigs. According to Dziuk and Henshaw (1958)

a single insemination resulted in a 40% conception rate and a 50% rate was obtained with insemination on two consecutive days. On the other hand, Holt (1956) and Akatov et al. (1962) observed no increase in conception rates from double matings. The latter, however, did observe a 2.2 increase in the average litter size when sows were serviced twice.

Inseminator differences.

An inseminator influence on fertility has been observed in dairy cattle (Dunn, 1961; Dombroske, 1964) and the same appears to occur in swine A.I. Two college veterinarians in a Norwegian Swine A.I. trial had a higher conception rate (70.2%) than 30 practicing veterinarians (57.9%). (Aamdal and Hogset, 1957). Madden (1959a), in a field trial of about 500 sows and 400 gilts, observed considerable variation in farrowing rates between technicians. For sows the rate ranged from 36% to 57%. One inseminator consistently produced a higher fertility. Since a sow rejection policy was in force it was concluded that this improvement represented a greater ability of this inseminator to detect estrus. Paredis (1961b) likewise observed differences of 28% to 49% in conception rates obtained by inseminators. A Wisconsin field trial (Self, 1961), with dairy technicians doing the breeding, resulted in inseminator conception rates which ranged from 71.4% to 14.3%. It was concluded that the technician is perhaps the most important single factor in the success of swine A.I. Madden (1960) estimated that conception rate improved from 40% to 70% when two swine technicians replaced 24 dairy inseminators.

Boar differences.

Aamdal (1959) and Paredis (1962) observed highly significant

($P < 0.01$) differences in conception rates between boars used for artificial insemination. The latter also noted highly significant ($P < 0.01$) differences in litter size between boars. Hancock and Hovell (1961) also noted a significant ($P < 0.05$) difference in fertility of two boars as judged by percent cleaved ova. Similar findings were indicated by Radford (1961a) and Madden (1959a). Bane (1959) reported that the fertility of stored semen differed with boars and First et al. (1963) detected a significant ($P < 0.05$) interaction between sperm age and boars. Earlier Wisconsin studies (Stratman and Self, 1961) indicated that conception rates between 7 boars did not differ appreciably. However, in a field trial with 11 boars (Self, 1961), a considerable range in conception rates between boars was observed, the spread being greater in gilt breedings than in sows. The ranking of the boars on the basis of conception rate in sows was almost in reverse order to their ranking in gilts.

Swine Artificial Insemination in Practice.

Experimental results compared to field results.

The preceeding literature review points out that considerable investigation into artificial insemination of swine has been conducted throughout the world. One striking characteristic of this research has been the differences in the results obtained in closely supervised experiments and those obtained in the field. DuMesnil du Buisson (1961), Campbell (1962) and Polge (1956a) reported conception rates in field trials to be as much as 20 to 30% below experimental results. In general, the lower rates were attributed to difficulty in accurately detecting

estrus.

This is not to imply that swine artificial insemination has not been successful in the field. On the contrary, satisfactory conception rates with large numbers of breedings were reported by Scandanavian, Japanese, French, Dutch, and Russian workers. This success was always accompanied by patience, diligence and persistance on the part of those working with the swine (Rowson, 1959). For example, in Asia where the manpower per sow is high, A.I. conception rates approached those obtained naturally. Similiarly, in closely supervised experiments throughout the world the conception rates from artificial insemination have often approached natural breeding.

Artificial insemination in Asia.

The countries where the largest number of swine are artificially inseminated each year are probably Japan and Russia. Unfortunately little is known of its actual extent in Russia, but in Japan, research in swine A.I. started in 1938 and by 1960 approximately 20% of the national pig herd was artificially inseminated (Niwa, 1958; Beattie et al., 1961). A farrowing rate of 68.2% was reported for 51,687 first inseminations in 1960. Some 500 breeding centers handle the inseminating, each center servicing small areas and on the average servicing only about 100 pigs annually. The artificial breeding of swine in Japan is an intensive, closely supervised program.

The Japanese A.I. techniques are also utilized in Formosa, Okinawa, and the Philippines. A successful experimental airlift of boar semen from Japan to Formosa was undertaken in 1954 (Niwa et al., 1959a). The

Philippines have had researchers working with swine for a period of years (Rodolfo, 1934a, 1934b; Noll, 1949; Coronel and Masankay-Arenas, 1954) but no large scale program has been developed. The latter workers have reported on the insemination of 271 sows resulting in a 24.4% conception rate. Carpenter (1962) indicated that a swine A.I. program was initiated in Thailand in February, 1961 and that approximately 500 sows were bred.

The present staus of artificial insemination on the Chinese mainland is difficult to ascertain. Felix Green, one of the few Westerners to recently tour China, states in his book, China (1961) that swine are being bred by artificial insemination and this method is preferred to natural breeding by the managers of the communes. The government has undertaken a large scale drive to double China's pig population but the role of A.I. in this drive was not defined.

Artificial insemination in Europe and the United Kingdom.

A number of European countries have been conducting research and field trials on artificial insemination of swine. It is difficult to ascertain the extent of swine A.I. in some countries since figures are reported only for particular experiments, rather than national averages. Some initial information on the extent of swine A.I. was presented at a colloquium in Paris in 1959. The first service farrowing results, as reported by researchers, were as follows:

Norway - 50%, 4 Centers; 6-7,000 annually (Aamdal, 1959)

Sweden - 59% on 637 sows; 48% on 260 gilts (Bane, 1959)

Belgium - 51% on 394 sows (Paredis, 1959)

Denmark - 54% on 382 sows; 34% on 260 gilts (Jakobsen, 1959)

England - 42% on 1,993 sows and gilts (Madden, 1959a)

Additional reports were made on research in England (Melrose and O'Hagan, 1959; Polge, 1959), France (DuMesnil du Buisson and Dauzier, 1959), Japan (Niwa et al., 1959a) and the United States (Self, 1959).

More recently, at the 4th International Conference of Animal Reproduction, Beattie, Field and Melrose (1961) were able to compile a report on the status of swine A.I. in a number of countries. They reported the following:

Country	Worker	No. of inseminations	Conception rate	Remarks
England	Madden	652	71.5%	strict selection
England	Melrose and O'Hagan	--	58%	12% rejection
France	DuMesnil du Buisson	--	54.9%	no selection
Holland	Smidt	4,467	60.3%	12 to 15% rejection
Belgium	Paredis	6,000	43%	no selection

The 71.5% and 58% reported by English workers was considerably higher than earlier results in their country. Madden (1959a) had previously reported a 42% conception rate when no sow rejection was employed at the time of breeding. Holt (1956) only obtained a 14% farrowing rate on 1,114 matings; however, he later (Holt, 1959) improved this to 24.4% on 544 first services. The work of many other researchers already cited, such as Polge, Radford, Hancock and Mann, has added immeasurably to our present knowledge of swine A.I.

The 54.9% obtained in France was an improvement over the 36% on over 1,000 sows earlier reported by the same workers (Dauzier and Du Mesnil du Buisson, 1958). DuMesnil du Buisson (1961) also reported a 51.3% farrowing rate on 4,338 inseminations made with semen stored up to six days. At the Roville Experiment Station a 56.6% conception rate was obtained on 1,373 first services in 1962. (Corteel, 1963). This was a considerable improvement over the previous year when the rate had been 36.2% on 3,606 first inseminations. Recently Dzuik (1962) also reported that four centers in France obtained a 70% conception rate on 11,000 sows and gilts bred artificially.

Dzuik (1962) also updated information on A.I. results in Holland indicating a 60 to 70% farrowing rate from 20 breeding centers in 11 Provinces. The technicians were following a program of rejecting animals not considered to be in a good standing estrus. It was anticipated that 60,000 inseminations would be made in 1962.

Artificial breedings of swine were performed for the first time in Finland in 1960 (Jokinen, 1961). A 60% pregnancy rate was reported for the 120 sows inseminated. Larsson (1960) gave notice of swine A.I. in 5 centers in Sweden in 1959. A conception rate of 77.6% was obtained on 911 sow first services and 73.3% on 205 gilt first services.

There have been several recent accounts of swine artificial insemination expanding to other countries. Cerne (1960) reported on the first successful attempts to inseminate pigs in Yugoslavia. Campbell (1962) described results in Jamaica. Ben Joseph and DuMesnil du Buisson (1961) collaborated in the successful insemination of pigs in Israel

with semen flown from France. Air transportation also has been used successfully to impregnate sows in Ceylon with semen from Britain (Jeganathan and Fernando, 1961). In December, 1956 the United States Department of Agriculture (1957) participated in an experiment in which swine semen imported from Norway to Beltsville, Maryland resulted in a 46% farrowing rate in the 24 sows bred.

Artificial insemination in the United States.

In this country there has been considerable interest in swine artificial insemination but the development has been slow since the original investigations of the Missouri station (McKenzie et al., 1935, 1938, 1939; Lasley, 1940; Lasley and Bogart, 1944). Research on the problems coincident with swine A.I. has been subject to study by the agriculture experiment stations in New York (Young et al., 1957; Turkheimer et al., 1958), Illinois (Dzuik, 1958a, 1958b, 1960, 1963; Smith and Nalbandov, 1958), Wisconsin (Bialy and Self, 1959; First et al., 1963; Self, 1959, 1960, 1962; Stevermer et al., 1961, 1962; Stratman et al., 1958, 1959, 1960, 1962; Stratman, 1961), Ohio (Hess et al., 1957, 1960; Wilson et al., 1963), Iowa (Lovell, 1958; Self, 1960, 1962), Minnesota (Gerrits et al., 1962; Graham, 1963; Niwa et al., 1962) and Michigan (First et al., 1960) but disappointing results have prompted few large scale field studies.

Eastern Iowa Breeders at Cedar Rapids and Iowa Breeders at Des Moines supported early swine field studies (Self, 1961). In 1959 and 1960 four bull stud cooperatives in Wisconsin (Badger Breeders Coop; East Central Coop; Southern Breeders Coop and Tri-State Breeders Coop)

financed one of the largest swine A.I. field trials to be undertaken in this country. Using the facilities and personnel of the Badgers Breeders Coop, 485 inseminations were performed on 162 farms in 6 northeastern Wisconsin counties by 30 dairy technicians. A total farrowing rate of 41% (first and second services) was obtained on sows with an average litter of 10.6 pigs and 35.4% on gilts averaging 9.0 pigs per litter. The first service rates were 47.2% for sows and 35.3% for gilts. Farrowing rates were compared by technicians, boars, time of insemination and condition of estrus as already reported.

As a result of this preliminary study an exploratory commercial swine insemination program was initiated by Tri-State Breeders Coop, Westby, Wisconsin. Beginning in April of 1963 they developed a Swine Division that supplies semen from four breeds to a three county area. Charging $5 for first service and $2 for repeats, the two fulltime swine technicians averaged 85 inseminations a month for the first year. They reported conception rates of 74.3% on a 40-60 day non-return basis and farrowing rates of 72.5% on the first 273 sows and gilts. (Tri-State Breeder Coop, 1964). On March 1, 1964 the second commercial swine stud was to begin operation under the direction of Badger Breeders Coop. A swine A.I. program in 10 townships in Door County, Wisconsin was planned, with a $5.00 service fee for insemination to one of 4 Hampshire boars (First, 1964).

Another large scale swine A.I. field study was conducted in central Ohio from January 1, 1961 to July 15, 1962 (Wilson et al., 1963; Wilson, 1964). A 20.8% conception rate was obtained on 1,079 first inseminations

by 32 technicians on 453 farms. The greatest variation in fertility was between inseminators and between boars. As in previous field studies the major problem was accurate estrus detection on the farm.

III. EXPERIMENTAL PROCEDURE

General

Boars and facilities.

The boars used for these artificial insemination studies were of the Yorkshire, Hampshire, Duroc and Chester White breeds. They were selected on the basis of performance testing, as well as on their individual merit, with the exception of several Yorkshires which were part of a University inbred swine project.

The boars were housed in a 22' x 85' barn constructed specifically as a boar stud. The barn contains boar pens, feed room, heated collection room and a semen processing laboratory. The boar section of the barn is open to the east and contains twelve 5' x 16' pens with concrete floors and wood partitions. Bedding was placed in the back of the pens with feed and automatic waterers available in the front. The boars were maintained on a high fiber, 17% protein nutritionally balanced swine ration. No exercise was provided for the boars and they remained in their pens except for the time when semen was collected. A careful program of boar hygiene was followed, including careful attention to foot trimming, detusking and internal and external parasite control. No visitors were allowed to enter the barn without special sanitary precautions.

A six foot alleyway in front of the pens led to a 12' x 14' collection room housing the "dummy" sow. In cold weather the collection room was heated to 50° to 60° F. to prevent any extreme temperature shock to semen.

Boar training.

Over a five year period 39 boars were placed in the stud and only 2 boars could not be trained to mount the dummy. One was a young boar that also experienced difficulty in naturally serving gilts. The second boar was a mature boar that would mate naturally but would not accept the dummy. He was eliminated because of distrust and meanness toward the herdsman. A third boar trained easily to the dummy as a yearling but after a period of use refused to mount the dummy. This boar was later found to also refuse natural matings.

Thus 37 boars were successfully trained to mount the dummy, which was a burlap and canvas covered piece of 10 inch metal pipe fitted with adjustable legs and boar foot braces. The training and collection of boars took place in the collection room, which was used only for this purpose. Originally, boars were collected off of "teaser" gilts in the collection area prior to collection off the dummy. However, since moving to the new facilities, 12 young boars and 2 mature boars have been trained to the dummy without the presence of "teaser" gilts. It is essential that the person handling the boars have patience, work quietly and gain their confidence. Once the boar entered the room and mounted the dummy the collection procedure was initiated.

Collection procedure.

Two collection procedures were followed. The first utilized an artificial vagina made from a piece of rubber hose $2\frac{1}{4}$ x 7" (radiator hose) to which a rubber squeeze bulb was attached. A rubber liner was placed through the hose, folded back and attached at both ends. Warm water

(52° C.) was placed in the space between the artificial vagina and the rubber liner. The squeeze bulb could provide either steady or pulsating pressure to the interior of the vagina. A 12" rubber cone was fitted to one end of the artificial vagina.

As the boar obtained an erection and mounted the dummy the operator placed the end of the artificial vagina over the penis and the boar began his thrusting motions. As the penis passed through the warm vagina and into the rubber cone pressure stimulation was applied with the squeeze bulb. When the boar's penis was well extended through the artificial vagina and into the cone the operator grasped the rubber cone, locking his hand on the spiral tip of the boar's penis. The thrusting motions ceased and the boar became tranquil as ejaculation proceeded. The semen was collected in fractions as it flowed from the end of the rubber cone. The presperm, post sperm and gel fractions were collected in a 1000 ml. plastic beaker and the sperm rich portion directly into a 250 ml. plastic beaker that was suspended in a 40° C. plastic water jacket. The plastic vessels were preferred because there was less breakage and they were found to be poor conductors of extreme environmental temperature variations.

A second method of collection was used on many boars. After the boar mounted the dummy the operator grasped the tip of the penis directly with his plastic gloved hand. With practice the tip of the penis could be locked between the thumb and the index finger and the ejaculate's flow easily directed into the beakers in fractions as above. The advantage of this method was that it required less preparation and

was more sanitary since throwaway plastic gloves were used.

Semen handling.

Immediately upon collection the sperm rich fraction was transferred to the heated (70° F.) processing room adjacent to the collection room. The semen was then filtered through two layers of cheese cloth (to remove any gel) into a graduated cylinder where the volume was recorded. The semen was then transferred to a 500 ml. plastic bottle suspended by means of a styrofoam float in a large thermostatically controlled water bath (38° C.).

Concentration determinations were made by removing a small semen sample with a hemocytometer pipet, diluting it in the standard manner with 3% acetic acid solution, and counting sperm cells in a hemocytometer counting chamber. Two concentrations determinations were made on each sample; the average was used to calculate dilution rate.

Final semen dilution was performed by adding the desired amount of prewarmed extender to the semen as it remained in the water bath. Since both the semen and extender were in the bath prior to dilution, temperature shock was minimized.

Motility was estimated by counting the percentage of total and progressive motility in the freshly diluted sample. Several drops of stock extender (without egg yolk) were placed on a microscope slide on a warming plate (38° C.). A small amount of diluted semen was added to the slide which was then quickly coverslipped and observed under a phase contrast microscope. Estimations of percentage motility were then made. While this procedure was subjective and open to some estimation

error, it was felt to be an adequate method of identifying unsatisfactory samples. Very few samples had to be rejected because of low motility.

Subsequent to motility estimates the diluted semen sample was removed from the water bath, identified, capped and placed at room temperature (20° C.) to begin the initial cooling process. After 10 to 20 minutes the diluted sample was transferred to either a refrigerator (5° to 8° C.) or styrofoam shipping cartons containing ice cans. The cooling rate of semen in the refrigerator did not exceed 10° C. an hour. Semen shipped in styrofoam boxes cooled at a slightly slower rate.

Insemination procedure.

The insemination procedure used was basically the same in all the fertility studies. The standard dose was 5×10^9 spermatozoa in a 50 ml. volume. Plastic dairy inseminating catheters (6mm x 1cc x 16") were used with the tips modified to facilitate entry into the cervix. A short piece of polyethylene tubing connected the catheter to a 50 ml. plastic syringe, which served as the semen injector. In most instances the semen was inseminated at refrigerator temperature.

Diluter.

The diluter of choice in the field trials and campus breedings was the standard egg yolk-sodium bicarbonate-glucose extender also found to be the most satisfactory by Dzuik (1958).

Stock Solution:

1000 ml. distilled water

30 gm. glucose

1.5 gm. sodium bicarbonate

1,000,000 units penicillin

1 gm. dehydrostreptomycin

The stock was prepared and stored in a glass stoppered bottle at 5° C. Just prior to use the final extender was prepared. This consisted of 70% of stock solution and 30% of egg yolk (by volume).

Plastic containers, glassware and rubber tubing were washed with a laboratory soap (Sparkleen) and rinsed in ethyl alcohol and then distilled water.

Boar Semen Preservation

Motility studies of stored boar semen.

A series of investigations were conducted on boar semen storage and its influence on spermatozoa motility. A number of diluters and preservation procedures were examined and the motility determined after various storage periods, up through 21 days. Since boar spermatozoa undergo anabiosis upon cold temperature storage (8° C.) it became necessary to develop standard reactivation procedures. The following procedure was adopted. The polyethylene bottles containing the stored semen samples were slowly inverted until the anabiosis layer was again in suspension. Pipets were used to withdraw 10 ml. aliquots which were placed into test tubes. These unstoppered test tubes were vigorously agitated on a mechanical shaker for $1\frac{1}{2}$ to 2 hours at room temperature (25° C.). The test tubes were suspended in a thermostatically controlled 37° C. water bath and motility determinations were performed.

The estimation of motility, while highly satisfactory for general semen evaluation, was not critical enough to detect small motility

differences between stored boar semen samples. Therefore, a method of motility determination was developed wherein the number of motile spermatozoa could be more accurately counted. The procedure used a standard hemocytometer pipet and counting chamber. The reactivated semen sample was drawn into a clean hemocytometer pipet, as in a concentration determination, and a prewarmed physiological solution (saline or stock boar extender without egg yolk) was used to complete the dilution. The diluted sample was placed onto a warm hemocytometer slide and the number of progressively motile, motile and dead spermatozoa, within the microscope grid, counted. A total of at least 100 spermatozoa were counted for each sample and the progressive and total motility calculated. This procedure, provided a grid against which spermatozoa could be counted, prevented coverslip pressure directly upon the sample and allowed a direct motility count rather than an estimation.

Fertility trials with stored boar semen.

The value of motility as an indication of fertility of stored boar spermatozoa is questionable, so a number of inseminations were undertaken to test fertility of semen stored 5 to 10 days.

The animals used for these trials were gilts from the Southern Michigan State Prison (S.M.S.P.) and Michigan State University (M.S.U.) swine herds. They were maintained on pasture lots at M.S.U. during this study and were examined for estrus twice daily with a vasectomized boar. The gilts were slaughtered 25 to 50 days post breeding and their reproductive tracts examined for pregnancy and/or gross anatomical abnormalities.

Semen samples were collected daily and subjected to storage treatments suggested by earlier motility studies. Samples were stored in sealed, 100 ml. or 250 ml. polyethylene bottles at 8° C. Motility determinations were made on the stored samples prior to insemination at estrus.

Field Trials

Animals and procedures at the Southern Michigan State Prison.

The field trial held at Southern Michigan State Prison (S.M.S.P.) consisted of 654 inseminations made from November 5, 1962 to October 30, 1963. The majority of these breedings were performed on gilts, only 47 of them being sows. The entire breeding program at the S.M.S.P. farm was converted to artificial insemination. The gilts used in this trial were selected by the herdsman as the regular replacement females for the Hampshire herd.

The gilts were placed in pastures in groups of approximately 25 and were self fed a balanced ration containing 16% protein. Estrus detection was carried out by trustee inmates each morning from 9 to 10:30 a.m. These workers varied in their dependability, thoroughness and experience. Boars were not used to verify estrus and those gilts determined to be in estrus were assigned to holding pens to await insemination.

The semen was collected at the Endocrine Research Unit's A.I. Stud three mornings a week. The diluted semen was shipped by bus in sealed 500 ml. plastic bottles, packed with ice cans in styrofoam shipping containers. The semen reached the prison within 4 hours and was used for insemination approximately 6 hours, 24 hours, 30 hours or over

48 hours after collection. Upon arrival the diluted semen was unpacked, the temperature recorded and then placed in a refrigerator at 10° C.

Insemination was performed by three men who were employees of the Prison; they had previously been trained in A.I. techniques at the M.S.U. Boar Stud. Gilts in estrus were driven into a specially designed breeding crate where they were inseminated and ear tagged. Some gilts were reserviced 24 hours later. All of the animals presented to the inseminators were artificially inseminated and no rejection system was employed.

The bred gilts were placed in large pastures during gestation and were transferred to the farrowing barn as parturition approached. These pastures were checked for recyling gilts, but apparently the inmates did not perform this task effectively.

The major problem in the S.M.S.P. trial was the identification of gilts in the program. Since the Prison had no previous identification system the gilts were ear tagged with large ($2\frac{1}{2}$ x 1 5/8"), colored, butyl rubber tags. These tags were easy to read at a distance and seemed ideal in this situation where different men were working with the hogs. Unfortunately the tags were easily pulled out and identification was lost on one third of the females inseminated. Thus, complete information was obtained on only 438 breedings.

Animals and procedures at the Michigan State University swine farm.

The University swine breeding program consists of three week breeding periods, six times a year. The present study included 10 breeding periods from March 1962 to October 1963 during which the

fertility resulting from natural and artificial matings could be compared. During this period 174 gilts and 200 sows were serviced; 254 by natural service and 120 by artificial insemination.

The animals used constituted all the gilts and sows serviced in the University Yorkshire, Hampshire and crossbred herds during the period of this study. All were handled in the same manner, irrespective of breeding method. There were 25 boars used during this period, 19 naturally and 6 artificially. Management and nutrition was approximately the same for both groups of boars.

The females were housed in outside lots, approximately 10 to 20 to a group, and were regularly checked for estrus once daily with a boar. Females bred naturally were hand mated to selected boars. Females mated artificially were serviced in the lots if they stood quietly. Occasionally a sow or gilt would not stand for insemination and these were placed in a specially constructed breeding crate for the insemination. Both ear notches and ear tags were available as a means of female identification.

IV. RESULTS

Boar Semen Preservation

Motility of stored boar semen.

The influence of various treatments on the progressive and total motility of stored boar spermatozoa was examined. A number of treatments, including changes in storage temperatures, diluents and atmospheric environment, maintained satisfactory sperm motility for periods of 5 to 14 days. The average progressive and total motilities of samples stored at 8° C. in the standard egg yolk-glucose-sodium bicarbonate diluter are presented in table 1. The term "pooled" samples refers to samples in which the semen from several boars was combined prior to dilution and storage. The "centrifuged" samples had the spermatozoa removed from their seminal plasma by centrifugation for 20 minutes at 1020 x G prior to resuspension and storage in the standard diluent.

TABLE 1

Progressive motility (PM) and total motility (TM) of boar spermatozoa after storage.

Treatment	No. samples	1 da.		5 da.		7 da.		10 da.		Best 7 da.	
		PM	TM	PM	TM	PM	TM	PM	TM	PM	TM
Standard diluter	112	72.7	86.8	25.5	65.3	20.1	54.3	23.3	47.5	61	84
Centri-fuged	63	69.1	79.3	41.1	76.2	25.4	58.1	14.4	48.3	67	83
Pooled	40	71.2	84.8	41.2	62.2	23.2	61.7	20.5	38.2	73	85

Fertility of stored boar semen.

Fertility tests of boar semen stored 5 to 11 days were conducted with 55 samples. Estrus was not observed in 5 of the 51 gilts used but the

remaining 46 were inseminated a total of 55 times, 9 of them being re-serviced when they returned to estrus. (A complete record of these inseminations appears in Appendix Table A). A number of the inseminations were performed with "pooled" and "centrifuged" samples. Several other treatments were also tested with 24 inseminations but no pregnancies resulted. A summary of the fertility results with stored boar spermatozoa is presented in table 2.

TABLE 2

The fertility of boar spermatozoa stored for 5 to 11 days.

Treatment	No. bred	No. preg.	Preg. rate	Av. no. of fetuses
Pooled	15	2[1]	13.3%	6.0
Centrifuged	16	3[2]	18.8%	6.3
Other	24	0	0	0
Total	55	5	9.1%	6.2

[1]Both samples stored 7 days.
[2]Samples stored 5, 6 and 7 days.

Southern Michigan State Prison (S.M.S.P.) Field Trial

Overall results.

The field trial at S.M.S.P. was inaugerated to establish the effectiveness of swine artificial insemination under field conditions, and to provide large numbers of gilt inseminations to effectively test experimental semen samples. The first objective was effectively accomplished, however, due to poor farrowing results insufficient numbers of gilts were available to test a variety of experimental semen samples. In the year from November, 1962 to October, 1963 a total of 654 insemi-

nations were performed. As already mentioned, considerable difficulty was encountered in identification of these gilts; thus complete information was available on only 438 of these breedings. The results of first and repeat breedings are summarized in table 3.

TABLE 3

Farrowing rate and average litter size of artificially inseminated swine.

Breeding	No. bred	Farr. rate	Litter size
First	276	34.8%	6.8
First repeat	115	27.0%	6.6
Second repeat	35	25.7%	8.5
Third or more repeat	12	33.3%	10.3
Total of all breedings	438	32.0%	7.0

These data were also summarized and examined to determine the influence of the number of breedings during estrus, inseminators, boars, storage age of semen, runback at breeding, number of spermatozoa inseminated, progressive motility, total motility and arrival temperature. Those animals serviced three or more times and any with missing data were not used in subsequent analyses, thus complete information is presented on 422 artificial inseminations.

The influence of number of services during an estrous period on A.I. fertility.

At the beginning of this trial approximately one half of the gilts were inseminated once and the other half were inseminated twice during estrus, the repeat services occurring 12 to 24 hours following the

initial breeding. This policy was continued until February 1963 when non-return rates indicated a much higher conception resulted from the double inseminations. Therefore the data on the number of services in a heat period were summarized for only the first 4 months and is presented in table 4.

TABLE 4

The influence of number of services during estrus on the fertility of artificially inseminated swine.

No. of services	No. bred	Farr. rate**	Litter size
Once	61	21.3%	6.9
Twice	92	44.5%	6.3

** $\chi^2 = 8.6$, significant at $P < 0.01$.

Influence of inseminator on fertility with swine A.I.

Three men performed the inseminations. In the instances when the pigs were serviced twice, different inseminators often performed each breeding; this situation is referred to as "combined" in table 5. The results by inseminator include females inseminated either once or twice by the same technician.

TABLE 5

The influence of different inseminators on fertility of artificially inseminated swine.

Inseminator	No. bred	Farr. rate**	Litter size
A	73	30.1%	6.9
B	145	28.3%	7.0
C	55	52.7%	7.3
Combined	149	28.2%	6.8

** χ^2 =12.48, significant at P< 0.01.

Influence of different boars on fertility with swine A.I.

Three purebred Hampshire boars were used throughout the study. The farrowing rates and litter sizes, by boar are presented in table 6.

TABLE 6

The influence of different boars on fertility of artificially inseminated swine.

Boar	No. bred[1]	Farr. rate**	Litter size
5-6	192	21.4%	6.4
11-11	108	36.1%	7.6
12-10	120	41.7%	6.9

** χ^2 = 16.11, significant at P < 0.01.
[1]Data on 2 gilts excluded as they were serviced by a fourth boar.

Influence of age on the fertility of stored boar semen.

The diluted semen was used either on the day of collection (6 hours), on the following morning (24 hours) or on the following afternoon

(30 hours). These three periods represented the stored age of the majority of the semen, but occasionally older semen samples were used (48 hour or older). In gilts serviced two times, calculations were based on the semen age at the time of the first insemination. No significant difference was found in the farrowing rate due to semen storage age. (Table 7).

TABLE 7

The influence of storage age of semen on fertility of artificially inseminated swine.

Stored semen age	No. bred	Farr. rate	Litter size
6 hours	306	32.0%	7.1
24 hours	58	30.5%	5.6
30 hours	44	30.0%	7.5
48 hours +(a)	14	7.1%	2.0

(a) 54 hour old semen was the oldest semen utilized that resulted in conception in this study.

Influence of semen backflow following insemination on A.I. fertility.

The inseminators were asked to make estimations of the amount of semen that flowed back out of the vagina following artificial breeding. While this was a purely subjective measurement it seems a cause for concern among both cooperating farmers and researchers. Therefore the relationship of backflow to farrowing rate and litter size was examined in the present study. Since many of the females were inseminated twice and exhibited different amounts of backflow at each breeding, only those were included that either (1) were inseminated once or (2) exhibited the

same backflow at both services. The conception rate was significantly higher ($P < 0.01$) when some backflow occurred after insemination as indicated in table 8.

TABLE 8

The influence of semen backflow on fertility of artificially inseminated swine.

Estimate of backflow	No. bred	Farr. rate**	Litter size
None	67	10.4%	7.0
Slight	39	43.6%	7.4
Medium	65	40.0%	6.9
Large	52	38.5%	6.1

** $\chi^2 = 55.59$, significant at $P < 0.01$.

Influence of number of spermatozoa inseminated on A.I. fertility.

The concentration and volume inseminated were generally held at 5×10^9 spermatozoa in a 50 ml. volume. However, on several occasions there was an abundance of semen at collection, so the concentration remained intentionally higher than 5×10^9. Also on a few occasions semen was extended to less than 5 billion spermatozoa per insemination sample. The volume of diluted semen inseminated was maintained at 50 ml. regardless of sperm concentration. A summary, by concentration, appears in table 9. The differences found were not significant but there were relatively few observations at concentrations other than 5×10^9.

TABLE 9

The influence of the number of spermatozoa inseminated on fertility of artificially inseminated swine.

Spermatozoa concentration	No. bred	Farr. rate	Litter size
4.0 - 4.9 x 10^9	21	42.9%	6.7
5.0 - 5.9 x 10^9	276	32.2%	7.1
6.0 - 6.9 x 10^9	57	24.6%	7.2
7.0 - 7.9 x 10^9	30	26.7%	5.5
8.0 - 8.9 x 10^9	21	9.5%	7.5
over 9.0 x 10^9	17	35.3%	6.7

Influence of progressive motility on semen fertility.

Progressive motility and total motility of spermatozoa are often used as estimates of semen quality. This relationship to fertility was examined in the S.M.S.P. trial. The motility estimates were made at the time of dilution of the semen and not at the time of insemination.

The data in table 10 suggest a decline in the farrowing rate at the lower progressive motility levels. These differences were nonsignificant by chi-square analysis.

TABLE 10

The influence of progressive motility of spermatozoa on fertility in artificially inseminated swine.

Progressive motility[1]	No. bred	Farr. rate	Litter size
20-29%	13	15.4%	5.0
30-39%	27	25.9%	6.7
40-49%	89	32.6%	8.3
50-59%	61	42.6%	7.8
60-69%	112	30.4%	5.7
70-79%	74	32.4%	7.3
80-89%	46	35.8%	5.8

[1]Motility estimates made at time of semen dilution.

Influence of total motility on semen fertility.

When the percent total motility was related to farrowing rate they appeared to increase together. (Table 11).

TABLE 11

The influence of total spermatozoa motility on fertility of artificially inseminated swine.

Total motility[1]	No. bred	Farr. rate*	Litter size
60-69%	36	13.9%	5.1
70-79%	48	29.2%	8.8
80-89%	209	37.3%	7.6
90-99%	129	31.0%	6.3

* $\chi^2 = 9.25$, significant at $P < 0.05$.

[1]Motility estimates made at time of semen dilution.

Influence of arrival temperature of semen fertility.

The temperature of the diluted semen sample was measured on arrival at the Prison. The arrival temperature varied considerably so the influence of arrival temperature on the subsequent fertilizing capacity of the semen was examined. Since the collection, dilution, transportation and pickup of semen were on a rigid schedule the arrival temperature should in effect be a measurement of semen cooling rate. This summary is presented in table 12.

TABLE 12

The influence of semen arrival temperature on fertility of artificially inseminated swine.

Arrival temp.	No. bred[1]	Farr. rate	Litter size
8° C.	19	42.1%	6.9
9° C.	31	45.2%	7.5
10° C.	55	25.5%	7.3
11° C.	34	14.7%	4.8
12° C.	18	38.9%	7.4
13° C.	45	42.2%	8.9
14° C.	52	32.7%	5.8
15-16° C.	40	37.5%	6.4
17° C.	14	21.4%	7.0
18-19° C.	14	28.6%	6.3
20-21° C.	29	20.7%	6.7

[1]Some data missing due to failure to record semen arrival temperature.

Comparison of fertility of artificially inseminated gilts and sows.

The majority of the matings were to gilts although a few first litter sows were included. The distinction between sow and gilts is referred to as female reproductive age in table 13.

TABLE 13

A comparison of fertility of artificially inseminated gilts and sows.

Female reproductive age	No. bred	Farr. rate	Litter size
Gilt	391	30.4%	6.8
Sow	47	44.7%	8.0

At this point a more sophisticated statistical analysis was made of the more salient factors influencing fertility in the S.M.S.P. swine A.I. trial. A least squares method of statistical analysis was employed because of the unequal subclass numbers. The analysis of variance was set up to include the effects due to number of services during estrus, inseminators, boars and semen storage age. Although differences due to semen age were not significant in the initial chi-square analysis this effect was included in order that it could be analyzed more critically.

The influence of semen backflow was not included since insufficient meaningful observations were available on the entire population. The number of spermatozoa inseminated, the motility and the arrival temperature were not included as components of the final analysis since prior examination had indicated they exerted little influence on A.I. fertility.

The information was punched onto IBM cards and the necessary least square computations were performed on a C.D.C. 3600 electronic computer.

The direct effect of these factors on the variance in farrowing rate is presented in the analysis of variance. (Table 14).

TABLE 14

Analysis of variance of factors influencing farrowing rate of artificially inseminated swine.

Source	df	Sum of squares	Mean square	F
Direct effect of number of services	2(a)	2.50	1.25	6.19**
Direct effect of inseminator	3	1.66	.55	2.74*
Direct effect of boar	2	2.77	1.29	6.36**
Direct effect of semen age	3	.63	.21	1.04
Error	411	83.10	.202	

* $P < 0.05$
** $P < 0.01$
(a) 1. bred once) until February 1963.
2. bred twice)
3. all breedings after February 1963.

This analysis indicates a highly significant ($P < 0.01$) effect on farrowing rate due to number of services during estrus and on the boar used. A significant ($P < 0.05$) effect was also indicated due to different inseminators. No significant influence was ascribed to the stored age of the semen at time of insemination.

A particular treatment effect was estimated by using the method of least square which were adjusted for other factors influencing farrowing rate. The least square estimate for two inseminations during estrus was 22% higher than for one. The farrowing rates for inseminator C was 16% above A and 20% above B. The farrowing rate of boar 5-6 was 19% below boar 12-10 and 14% below that of boar 11-11.

The influence of these same factors on litter size of the 136 farrowings was analyzed in a similar manner and is presented in table 15.

TABLE 15

Analysis of variance of factors influencing litter size of artificially inseminated swine.

Source	df	Sum of squares	Mean square	F
Direct effect of number of services	2	26.07	13.04	1.81
Direct effect of inseminator	3	2.16	.72	.10
Direct effect of boar	2	35.03	17.51	2.44
Direct effect of semen age	3	22.95	7.65	1.06
Error	125	898.94	7.19	

None of the factors examined in the S.M.S.P. study had a significant effect on litter size in the females which did farrow.

Michigan State University Swine Farm Trial

A comparison between natural and artificial mating at M.S.U.

The breeding program at the University swine farm afforded an opportunity to compare natural and artificial mating under identical management practices within the same swine herd. Farrowing rates and litter sizes were available on 374 females serviced from March 1962 to October 1963.

Comparisons between type of mating (natural vs. artificial), female reproductive age (sows vs. gilts) and number of services during estrus (once vs. twice) are summarized in table 16.

TABLE 16

A comparison between natural and artificial matings in the MSU swine herd.

Type of breeding	NATURAL BREEDING			ARTIFICIAL INSEMINATION		
	No. bred	Farr. rate	Litter size	No. bred	Farr. rate	Litter size
Gilts bred once	49	73.5%	9.2	13	38.5%	7.4
Gilts bred twice	71	57.7%	8.5	41	56.1%	8.4
Total gilts	120	64.2%	8.8	54	51.9%	8.2
Sows bred once	27	77.8%	11.8	35	42.9%	9.7
Sows bred twice	107	61.7%	10.7	31	54.8%	10.8
Total sows	134	64.9%	11.0	66	48.5%	10.3
Total breedings	254	64.5%*	10.0	120	50.0%*	9.3

* $\chi^2 = 6.84$, significant at $P < 0.01$.

Influence of boars on fertility in naturally and artificially mated swine.

An examination of the data by boars is presented in tables 17 and 18. A significant difference ($P < 0.05$) in farrowing rate was observed between boars used both naturally and artificially.

TABLE 17

The influence of different boars on fertility of naturally bred swine.

Boar no.	No. bred	Farr. rate*	Litter size
1	53	66.0%	9.8
2	13	53.8%	8.3
3	20	100%	11.3
4	43	58.1%	10.2
6	24	75.0%	9.7
7	14	57.1%	9.5
16	48	72.9%	9.1
Others (1)	39	41.0%	10.6

(1) Combination of 12 boars that had less than 10 breedings.
* $\chi^2 = 14.65$, significant at $P < 0.05$.

TABLE 18

The influence of different boars on fertility of artificially inseminated swine.

Boar no.	No. bred	Farr. rate*	Litter size
11	24	29.2%	8.9
13	25	52.0%	8.3
19	35	48.6%	10.3
20	22	50.0%	8.5
Other (1)	14	78.6%	10.5

(1) Combination of 2 boars that had less than 10 breedings.
* $\chi^2 = 10.1$, significant at $P < 0.05$.

The influence of breeding season on fertility of naturally and artificially mated swine.

The breeding program at MSU is based upon six farrowings a year. Therefore, the breeding schedule consists of 6 three week breeding periods. A summary of the 10 breeding periods occurring during this study are presented in table 19.

TABLE 19

The influence of breeding period on fertility of naturally and artificially mated swine.

Breeding period	Natural breeding			Artificial insemination		
	No. bred	Farr. rate	Litter size	No. bred	Farr. rate	Litter size
March 21 to April 10, 1962	37	64.9%	9.8	--	--	--
May 21 to June 10, 1962	34	76.5%	11.2	--	--	--
July 21 to Aug. 10, 1962	29	79.3%	10.5	13	73.8%	9.0
Sept. 20 to Oct. 11, 1962	22	68.2%	7.7	5	0	0
Nov. 20 to Dec. 12, 1962	25	48.0%	7.5	26	65.4%	8.4
Jan. 18 to Feb. 13, 1963	24	79.2%	10.5	19	60.5%	9.3
March 20 to April 10, 1963	15	66.7%	9.1	26	50.0%	8.8
May 21 to June 10, 1963	30	60.0%	10.6	11	54.5%	11.8
July 21 to Aug. 10, 1963	18	22.2%	11.0	12	50.0%	10.2
Sept. 23 to Oct. 14, 1963	20	65.0%	10.1	8	37.5%	10.7

As in the previous trial the roughness of the chi-square analysis with unequal subclass numbers suggested the need for a more complete statistical analysis. Therefore, these data were also subjected to least squares analyses with a C.D.C. 3600 electronic computer. Table 20 contains the analysis of variance of factors affecting farrowing rate.

TABLE 20

Analysis of variance of factors influencing farrowing rate in naturally and artificially mated swine.

Source	df	Sum of squares	Mean square	F
Direct effect of type of mating	1	.70	.70	3.18
Direct effect of reproductive age	1	.14	.14	.64
Direct effect of no. of services	1	.06	.06	.27
Direct effect of boars/type of mating	11	6.21	.56	2.55**
No. of services x type of mating interaction	1	.93	.93	4.23*
Direct effect of breeding seasons	9	2.88	.32	1.45
Error	349	76.78	.22	

* $P < 0.05$
** $P < 0.01$

The boar used was the only factor examined that had a highly significant effect ($P < 0.01$) on farrowing rate. The chi-square analysis had indicated a significant effect due to type of mating, but the difference in farrowing rates of naturally (64.5%) and artificially (50.0%) mated swine was nonsignificant by the analysis of variance. When these data were adjusted for all factors besides type of mating the least square estimate of the difference was 12%.

The analysis of variance also indicated a significant interaction ($P < 0.05$) between the number of services during estrus and the type of mating. The least squares estimate of this interaction is presented in table 21.

TABLE 21

Farrowing rates by type of mating and number of services during estrus.

		Type of mating		
		Natural	Artificial	Average
No. of services	1 x	69%	43%	56%
	2 x	59%	61%	60%
	Av.	64%	52%	

An analysis of the factors influencing litter size of those that farrowed is presented in table 22.

TABLE 22.

Analysis of variance of factors influencing litter size of naturally and artificially mated swine.

Source	df	Sum of squares	Mean square	F
Direct effect of type of mating	1	4.06	4.06	.42
Direct effect of reproductive age	1	118.01	118.01	12.15**
Direct effect of no. of services	1	16.13	16.13	1.66
Direct effect of boars/type of mating	11	114.04	10.37	1.07
No. of services x type of mating int.	1	53.75	53.75	5.53*
Direct effect of breeding season	9	218.37	24.26	2.50**
Error	199	1,932.52	9.71	

* $P < 0.05$
** $P < 0.01$

The reproductive age and the breeding season both had highly significant ($P < 0.01$) effects on litter size. The least squares estimate in-

dicated that sows averaged 1.96 more pigs per litter than gilts when the data was adjusted for the effects of all other factors.

The interaction between the number of services during estrus and the type of mating also was significant ($P < 0.05$) and the least squares estimate of this interaction is presented in table 23.

TABLE 23

Litter size by type of mating and number of services during estrus.

		Type of mating		
		Natural	Artificial	Average
No. of services	1 x	9.85	7.89	8.87
	2 x	9.17	10.29	9.73
	Av.	9.51	9.09	

The MSU results on swine A.I. were analyzed independently of the natural breedings in order that they could be compared with the S.M.S.P. swine A.I. results. Backflow was recorded on 109 of the artificial breedings and the farrowing rates and litter sizes were compared. These differences, as presented in table 24 were not significant.

TABLE 24

The influence of backflow at insemination on fertility of artificially inseminated swine.

	No. bred	Farr. rate	Litter size
No backflow	61	54.1%	9.42
Some backflow	48	45.8%	9.18

Table 25 includes the least squares analysis of factors influencing farrowing rate in M.S.U. artificially inseminated swine. None of the factors examined significantly influenced farrowing rates.

TABLE 25

Analysis of variance of factors influencing farrowing rate in artificially inseminated swine.

Source	df	Sum of squares	Mean square	F
Direct effect of reproductive age	1	.16	.16	.67
Direct effect of number of services	1	.06	.06	.25
Direct effect of boars	4	1.89	.47	1.96
Direct effect of seasons	7	1.67	.24	1.00
Error	106	25.48	.24	

The data was also examined for factors influencing litter size (Table 26).

TABLE 26

Analysis of variance of factors influencing litter size in artificially inseminated swine.

Sources	df	Sum of squares	Mean square	F
Direct effect of reproductive age	1	49.49	49.49	4.90*
Direct effect of no. of services	1	1.57	1.57	.15
Direct effect of boars	4	111.26	27.82	2.75*
Direct effect of season	7	141.28	20.18	2.00
Error	46	464.87	10.11	

* $P < 0.05$

The reproductive age (sow or gilt) and the boar used were the only factors that significantly influenced litter size in the artificially

inseminated swine on the M.S.U. farm. The least square estimate of difference in litter size due to reproductive age indicated that at M.S.U. artificially inseminated sows averaged 2.1 more pigs per litter than gilts.

V. DISCUSSION

Boar Semen Preservation Trials

The inability of boar spermatozoa to maintain fertility after periods of storage is one of the major problems confronting swine artificial insemination. A "shotgun" approach to the problem of storage was undertaken and a number of treatments were found that maintained motility for extended periods. In fact, dilution with the standard extender and refrigeration at 8° C. in sealed polyethylene bottles for 10 days maintained 50% to 60% total motility consistently.

An unsatisfactory relationship between motility and fertility of stored boar spermatozoa was noted by Stratman et al. (1958) and Stevermer et al. (1964). It seemed advisable, therefore, to conduct fertility trials with boar semen stored at least 5 days. Primarily two treatments prior to storage were examined: (1) separation of spermatozoa from their seminal fluids by centrifugation and (2) pooling the ejaculates of two boars.

Stratman (1961) observed that spermatozoa survival in storage was best when they were removed from their seminal fluids. At M.S.U. this was attempted by centrifugation or by separation after anabiosis using a separatory funnel. No pregnancies were obtained from 4 breedings with semen treated in the latter manner but a 18.7% rate was obtained on 16 breedings with 5 to 7 day old centrifuged semen. Bialy and Self (1959) and Self (1959) reported that removal of accessory fluids by centrifugation significantly lowered the motility of boar spermatozoa after

two days storage but no fertility tests were conducted. In the present study motility was not adversely affected in the centrifuged sample as compared to the control.

The use of pooled ejaculates has increased the fertilizing capacity of semen in cattle (Hess et al., 1954), sheep (Hamraev, 1958) and rabbits (Beatty, 1960). Individual ejaculates were compared with pooled ejaculates in swine by First et al. (1960) but 64 gilt breedings indicated no difference in farrowing rates due to pooling. In the present study a 13.3% pregnancy percentage was obtained from 15 inseminations with pooled semen samples that had been stored 5 to 10 days.

A total of 55 inseminations with boar semen stored 5 to 11 days resulted in a 9.1% pregnancy rate when all experimental treatments were combined (Table 2). The average number of fetuses 25 to 50 days post breeding was 6.2. DuMesnil du Buisson (1961) obtained a 45.4% conception rate from 33 inseminations with semen stored 4, 5 or 6 days at 15° C. under a CO_2 atmosphere. This compared to a 53.4% rate for fresh semen in the same study. However other workers have indicated that fertility generally decreases drastically after the first day of storage. Conception rate was reduced one half by storing boar spermatozoa over 24 hours (Holt, 1959). Aamdal and Hogset (1957) observed farrowing rates of 79.2% for fresh semen, 60.9% for 1 day, 36.5% for 2 day, 16.1% for 3 day and 5.6% for 4 day old boar semen. Polge (1959) obtained farrowing rates of 72%, 42%, 28%, 17% and 13% for semen stored for the same periods. The pregnancy rates obtained in the present semen

preservation trials were no better then those obtained in these previous studies.

Swine A.I. Field Trials

The farrowing results from artificial insemination of swine at S.M.S.P. were disappointing, but still provided considerable information on some of the factors that influence fertility following A.I. The first service farrowing rate (34.8%), although comparable to the 20.8% rate in an Ohio trial (Wilson et al., 1963, Wilson 1964) and the 35.3% reported on first service gilts in Wisconsin (Self, 1961), was considered insufficient for widespread practical acceptance. On the other hand, the comparison between natural and artificial breedings on the M.S.U. swine farm indicated that under similiar conditions the conception rate for natural mating (64.5%) was not significantly different from the conception rate for artificial mating (50.0%). Subin (1962) and Stratman (1961) noted that conception rate of artificially mated sows did not differ appreciably from sows bred naturally. Lovell (1958) obtained a 63% conception rate on 40 sows serviced artificially while 75% of the naturally bred control sows conceived. A similar comparison by Paredis (1961b) also indicated that conception rates for artificially inseminated swine are generally lower than those bred naturally. Madden (1959a) obtained an 86% farrowing on sows bred naturally but when the same sows were serviced artificially in the next breeding season only 45% farrowed. The differences in litter sizes (0.5 pig) were not significant and indicated that litter size is little affected by the type of mating. In the present study an 0.7 pig difference in favor of natural mating was

observed in the M.S.U. herd. This was not significant and approached the nonsignificant 0.6 pig difference observed by Paredis (1961b).

A slight decrease in the farrowing rate of repeat breedings was noted in the S.M.S.P. study (table 3). This difference was not significantly below that for first services. This characteristic between first and repeat services has been observed previously by other researchers. Madden (1959a) noted little difference in conception rates for repeat inseminations. A 24.4% conception rate for first services and a 24.0% for repeat services was recorded by Holt (1959). Similarly, Self (1961) found the rates for second services comparable to first services in both sows and gilts. Jacobsen (1959) obtained a 34.2% farrowing rate on first service gilts which was significantly below ($P < 0.05$) the 46.5% rate for repeat breedings. It appears that when the farrowing rates are low, as they have been in swine artificial insemination, there is little selection for fertility in the first breedings. Therefore the S.M.S.P. data for first and repeat inseminations were combined in further statistical analyses.

Two services during estrus significantly ($P < 0.01$) increased conception rate in swine artificially inseminated at S.M.S.P. This did not occur in the M.S.U. trial. However, there was a significant interaction ($P < 0.05$) between the number of services and the type of mating. The least squares estimates of farrowing rates, as presented in table 21, suggest that the number of matings did not greatly influence farrowing rate in the combined data because of this interaction. Surprisingly, at M.S.U. the use of two matings decreased the conception rate 10% in

naturally mated sows and gilts. On the other hand, the sows and gilts artificially inseminated twice during estrus had an 18% higher farrowing rate than those inseminated only once. Therefore, both the S.M.S.P. and M.S.U. studies indicate that A.I. farrowing rates are improved with two inseminations. The trend towards increased conception with double artificial services of swine was noted 25 years ago by McKenzie et al. (1939), when they obtained a 52.9% conception rate with double inseminations and a 20.7% rate with single inseminations. Weaver and Bogart (1943) also reported an 18% increased conception with double inseminations. A 10% improvement in conception was indicated by Dziuk and Henshaw (1958) when swine were inseminated twice during estrus.

At M.S.U. concurrent with the decreased conception of swine mated naturally twice during estrus was a decrease in the average litter size. Litter size was also subject of a significant interaction ($P < 0.05$) between number of services and type of mating (Table 23). Alexandrowicz (1955) observed that litter size was increased by double matings. At M.S.U. the A.I. swine that were serviced twice averaged 2.4 more pigs than those inseminated once. The doubly mated gilts at S.M.S.P. averaged 0.6 less pigs per litter so no consistent trend was evident. There is little reason to assume that two matings are more satisfactory than one breeding at the optional time of estrus. However, optimal time for service is difficult to ascertain when utilizing A.I. Madden (1959b) has stated that the greatest improvement in fertility in swine A.I. was due more to critical assessment of the optimum service time than any improvement in technique. The personnel and

methods employed at M.S.U. undoubtedly resulted in more accurate detection of estrus. Therefore, the differences in the two trials could be a reflection of the accuracy of detection of estrus.

The estrus detection at S.M.S.P. was not above criticism. It was conducted by inmates utilizing visual and manual pressure techniques without the assistance of a boar. These men often lacked an interest and desire to do their job well. In the Literature Review the importance of accurate estrus detection and correct timing of the breeding was repeatedly emphasized. No rejection of females for poor estrus was practiced by the S.M.S.P. inseminators. Another reason to question the accuracy of S.M.S.P. estrus detection was the high number of gilts (8.3% of total) that were reserviced or slaughtered for presumably returning to estrus and later found to be pregnant from earlier matings. In the M.S.U. trial pregnant gilts were not observed to recycle.

The different inseminator's influence on fertility led Self (1961) to conclude that perhaps the technician is the most important single factor in the success of swine A.I. Certainly the results from S.M.S.P. demonstrate a considerable range in the conception rates obtained by the three inseminators (28.3% to 52.7%) (Table 5), a difference significant at the 5% level. Similar ranges in the conception rates of inseminators were noted by Aamdal and Hogset (1957) where college personnel had a 70.2% rate as compared to 57.9% for 30 practicing veterinarians. Madden (1959b) also examined the inseminator influence on swine fertility. One technician was consistently higher than the others. This was attributed to his greater ability to assess the optimal stage of

estrus for A.I. Wilson et al. (1963) explained inseminator differences on the basis of experience. Since the conception rates did not appear to be markedly different after the inseminator had made 10 or more inseminations.

Statistical analysis revealed no significant inseminator effect on litter size but there was a trend for the best technician to have the highest litter size and the poorest the lowest. Madden (1959b) also noted that the best inseminators tended to have the largest litters but the difference was not significant. Of coincident interest was the attitude of the S.M.S.P. inseminators toward the swine A.I. project. Technician C, with the highest conception rate, had enthusiastically welcomed the trial and was partly responsible for its initiation as a research project. In addition this man was the swine herdsman and enjoyed working with swine. The other two technicians, although not swine men, accepted the assignment as part of their job; however, as the trial progressed it became apparent that inseminator B was discouraged and impatient with the A.I. program. No inseminator comparison was possible at M.S.U. because one highly qualified technician did the breedings.

The one factor in each study that consistently had the greatest influence on farrowing rates was the boar used. At S.M.S.P., where 3 boars were used, the range in farrowing rate was 21.4% to 41.7% while at M.S.U. the range on 6 boars was 29.2% to 78.6%. In the naturally mated swine the boar's range in farrowing rate was 41% to 100%. These differences in farrowing rates by boars were significant at the 1% level.

There were significant ($P < 0.05$) boar differences in litter size in the A.I. swine at M.S.U. Paredis (1962) reported highly significant ($P < 0.01$) differences in conception rates and litter size by different boars used for A.I.

Aamdal (1959) observed a statistically significant difference in conception rate between 16 boars used in over 6,500 inseminations. The conception rates by boars ranged from 37.5% to 60.8%. A range in farrowing rates by individual boars was also noted by Madden (1959a) but the differences were not significant. The difference in conception rate between high and low boars was greater in gilt than sow breedings, but the ranking of the boars on the basis of conception rate in sows was almost in the reverse order to their rank in gilts (Self, 1961).

Numerous reports on the effects of semen storage on farrowing rates and litter size are cited in the Review of Literature. The development of satisfactory storage methods for boar semen is the major problem limiting the expansion of swine A.I. A progressive nonsignificant decline in conception when semen age increased up to 24 hours was reported by Paredis and Vandeplassche (1962). A similar situation was noted by Aamdal (1959). The same observation was made in the present S.M.S.P. field trial where the farrowing rate of semen stored 6 hours (32.0%) was not significantly greater than that of semen stored 24 hours (30.5%) or 30 hours (30%). Litter size was also apparently unaffected by semen storage. The highest average litter sizes (7.5 pigs) occurred in gilts bred with 30 hour semen. Since only a limited number of breedings (14) were made with semen stored 48 hours or longer it is not possible to

formulate conclusions for storage periods longer than 30 hours. However, as already noted a marked decrease in fertility of semen stored longer then one day has been reported. With semen stored only 10 hours Jacobsen (1959) indicated that decreased farrowing rates and litter sizes with increasing semen age were significant. In his study semen stored 2 hours provided a 63.0% farrowing rate and an average of 11.3 pigs while semen 8 to 10 hours old resulted in a 48% farrowing rate and an 8.8 pig litter average. Conception did not result when 5 sows were inseminated with 12 hour semen. Madden (1959a), however, determined no evidence of a decrease in fertility with increasing semen age up to 10 hours. In the current S.M.S.P. trial semen was stored for 30 hours without a significant decrease in fertilizing capacity.

Semen backflow may occur following the artificial insemination of swine. Lasley and Day (1960) reported difficulty in making the semen remain in the female tract, and Hess et al. (1960a) suggested that backflow indicated improper insemination technique. Dzuik and Henshaw (1958), Madden (1959a) Radford (1961b) and Paredis (1961b) reported no relationship between backflow and fertility. This apparently occurred in the present trial with the M.S.U. swine herd, but the S.M.S.P. trial indicated conception was significantly higher ($P < 0.01$) when semen backflow occurred. Hammond (1957) and Polge and Rowson (1956) suggested that uterine contractions play an important role in sperm transport. It appears that semen backflow reflects muscular activity of the female reproductive tract. If this is correct backflow may be an accurate indication of proper uterine receptivity. On the other hand, backflow

could also result if the infusion of semen is too rapid, since considerable backflow occurs when large volumes of cold semen are inseminated too quickly. The quiet, deliberate insemination procedures utilized could explain why backflow was not related to increased fertility in the M.S.U. herd. Some difficulty was encountered in training the S.M.S.P. technicians to slow the speed of semen insemination. Of the swine inseminated at M.S.U. only 44% exhibited backflow as compared to 84% for those inseminated at the S.M.S.P. In each study backflow occasionally occurred even when the gilts were handled quietly and inseminated slowly. The relationship of semen backflow to fertility in these studies demonstrates that backflow does not indicate ineffective insemination technique. It is also true that backflow is sometimes observed following natural mating.

The complex relationship between the volume and spermatozoa concentration of the insemination dose has been discussed. The present trials were not designed to study this relationship. The concentration (5 billion) and volume (50 ml.) selected were within those levels recommended by other workers. (Stratman and Self, 1960; First, et al. 1960; Paredis, 1961b).

The most satisfactory test of boar semen quality is fertility. However, numberous studies have utilized spermatozoa motility as the end point in spermatozoa evaluation. The S.M.S.P. study indicated that progressive motility estimates made at the time of semen collection had little relationship to fertility except at the lower motilities. The lowest farrowing rate and litter size were obtained from the semen

samples with the lowest progressive motility, and the lowest total motility. Thus while motility estimates may not be closely related to fertility at higher motility levels it appears that poor motility reflects inferior spermatozoa. This is in support of earlier observations by Paredis and Vandeplassche (1962), Hess et al. (1960a), Ito et al. (1948b) and Niwa et al. (1959a) where higher conception rates were obtained when the initial motility exceeded 70%. There is some evidence which indicates that motility may be a particularly poor measure of fertility of stored boar spermatoza (Stratman et al., 1958; Self, 1959; Stevermer et al., 1964). This belief was also supported by previous, unpublished work at M.S.U.

A study of the cooling rate of boar spermatozoa (arrival temperature) was not intentionally designed into the S.M.S.P. study but the differences that did develop in shipment did not significantly influence the subsequent fertility of artificially inseminated swine. However, the arrival temperatures were within the range of those commonly accepted for storage of boar spermatoza (8° C. to 20° C.). Fertility apparently decreased when semen was received at the higher temperatures (17° C. to 21° C) but the results were inconsistent and no definite trend was apparent. For example the highest farrowing rate (45.2%) occurred from the 9° C. samples and the lowest (14.7%) from the 11° C. samples.

The study at M.S.U. afforded the opportunity to compare the fertility between sows and gilts. Generally, the farrowing rates are reported to be higher for sows than for gilts (Jacobsen, 1959; Self, 1961). No difference was observed in the present trial. The conception rate for

174 gilts was 60.3% as compared to 59.5% for 200 sows. The type of breeding (A.I. or natural) did not change this relationship between gilts and sows. A limited number of sow breedings at S.M.S.P. (10% of the total breedings) indicated a 30.4% farrowing rate for gilts and 44.7% for sows. In the M.S.U. study the average litter size was increased by more than 2 pigs in sows compared to gilts regardless of type of mating. This increase in average litter size of sows as compared to gilts was highly significant ($P < 0.01$).

The breeding season did not significantly effect farrowing rate and no particular trend was observed. The two highest farrowing rates for naturally bred sows occurred in July-August 1962 (79.3%) and January-February 1963 (79.2%). The lowest farrowing rate was in July-August 1963 (22.2%). There was a highly significant difference ($P < 0.01$) in litter size by seasons in the combined M.S.U. data but this difference did not exist when the A.I. results were analyzed alone. In several breeding periods the A.I. farrowing rate was superior to the rate for naturally bred swine.

The behavior pattern of swine during insemination may be important. DuMesnil du Buisson (1961) obtained a 63.8% farrowing rate when sows were "very quiet" at the time of insemination and 34.1% when they were "agitated". At the S.M.S.P. the procedure of driving the gilts into the breeding crate often had them in an agitated condition. The gilts were subjected to eartagging immediately prior to their first insemination, undoubtedly a stressful exposure. Patient handling of swine at breeding was recommended by Polge and Rowson (1956). Since it is recognized that

adrenalin will antagonize the normal activity of the female genital tract during breeding.

Gilts that failed to conceive after three inseminations were autopsied at the S.M.S.P. slaughtering plant and their reproductive tracts were examined. The incidence of anatomical abnormalities in the reproductive tracts of these gilts was not unusually high. Only 20.8% of the "non breeders" slaughtered exhibited reproductive abnormalities on gross examination and this represented less than 2% of all the gilts utilized in this trial.

Non return rates when utilized as a measure of swine fertility, are not satisfactory. The non return rate calculated for first breedings at S.M.S.P. was 51.7% while the farrowing rate for the same group was 34.2%. The same situation has been reported by Holt (1956) where a 71% non return rate represented a 14% farrowing rate in artificially inseminated swine.

In the S.M.S.P. trial a total of 61 litters, averaging 7.1 pigs, were farrowed to gilts that lost their ear tags subsequent to service. These unidentified farrowings approximated 29.4% of the total matings to animals that lost their tags after insemination. This approaches the 32% rate obtained in the tagged gilts and suggests that the loss of ear tags was random with respect to the various parameters of the trial.

A limited field trial was also conducted at the Ionia Reformatory farm in Ionia, Michigan in May 1963. Only 7 gilts and 13 sows were inseminated but this represented the entire spring breeding on the Reformatory farm. The sows and gilts were checked daily by an inmate

with a boar. Sows were inseminated on their first post weaning estrus and the gilts were serviced whenever they exhibited estrus during the three week breeding period. A breeding crate was used for the inseminations. The techniques used were similar to those used in other trials. The semen was provided from the Endocrine Research Unit Boar Stud and the insemination was performed by the dairy cattle herdsman and his assistant. The farrowing rates on first breeding for the 13 sows was 61.5% with an 8.25 pig average. Identification was lost on all of the gilts, but 2 farrowed an average of 4.5 pigs for a 28.6% farrowing rate. Although relatively few animals were inseminated, trends paralleled the S.M.S.P. trial in respect to the influence of number of services, inseminator, flowback and different boars on farrowing rates.

VI. SUMMARY AND CONCLUSIONS

Research on artificial insemination of swine was intiated at Michigan State University in 1959. A boar stud was organized and swine insemination techniques were investigated.

A series of boar semen preservation studies indicated that spermatozoa motility could be satisfactorily maintained with a variety of treatments for storage periods of 5 to 10 days. Fertility trials provided 55 inseminations with semen stored 5 to 11 days. The pregnancy rate at 25 to 50 day post breeding slaughter was 9.1% with an average of 6.2 fetuses per pregnancy.

Two experimental field trials were conducted to study the factors which affect fertility in swine artificial insemination. One trial, undertaken at the Southern Michigan State Prison farm, afforded complete information on 438 inseminations. The second trial, conducted on the Michigan State University swine farm, consisted of 120 artificial matings with 254 natural breedings for comparison. The overall conception rate on 276 first inseminations at S.M.S.P. was 34.8% with a 6.8 pig average litter size. The conception rate on 120 first inseminations at M.S.U. was 50.0% with a 9.3 pig average.

The farrowing rate for the naturally bred swine at M.S.U. was 64.5% while the rate for the artificially inseminated swine was 50.0%. This difference did not prove to be statistically significant when analyzed by the method of least squares. The average litter size for naturally bred swine was 0.7 of a pig higher than for those artificially inseminated, but this difference was not significant.

The conception rates obtained from first and repeat inseminations

at S.M.S.P. were not statistically different; however the number of inseminations during estrus significantly influenced farrowing rate. When gilts were serviced twice during estrus the farrowing rate was 44.5% as compared to 21.3% for gilts serviced once. This difference was highly significant ($P < 0.01$). An increase in farrowing rates of swine inseminated twice during estrus was also apparent in the M.S.U. trial but the differences were not significant. There was a significant interaction ($P < 0.05$) between type of mating and number of services with the naturally bred swine having an increased farrowing rate in favor of the singly bred gilts and sows.

The age of semen up to 30 hours had no influence on fertility in the S.M.S.P. study, but the inseminator and the backflow of semen at insemination markedly influenced farrowing rates. Semen stored 6 hours, 24 hours, 30 hours and a few samples stored over 48 hours did not significantly influence litter sizes or farrowing rates. When three inseminators were compared their differences in farrowing rates were significant ($P < 0.05$). An estimate of semen backflow during and after insemination was related to farrowing rate, the farrowing rate being significantly ($P < 0.01$) higher when some backflow occurred. No such relationship was observed in the M.S.U. studies.

The greatest factor to influence fertility at S.M.S.P. and M.S.U. was the differences between boars. A highly significant ($P < 0.01$) boar effect on farrowing rate was observed in both studies. At M.S.U. this difference was significant for boars used either naturally or artificially.

Sows did not have a significantly higher farrowing rate than gilts following natural or the artificial matings at the University. The average litter size of sows, however, was significantly ($P < 0.01$) larger than gilts. The majority of the breedings at S.M.S.P. were with gilts. However, the few sows that were artificially inseminated had higher farrowing rates and larger litters than gilts. These differences were not significant.

The season of breeding did not significantly influence farrowing rate in the M.S.U. breedings although the breeding season influence on litter size was highly significant ($P < 0.01$).

Motility estimates, at the time of collection and dilution of the semen, were not closely related to fertility. However, the lower progressively motile samples resulted in the lowest fertility and samples with less than 70% total motility were significantly ($P < 0.05$) lower in fertility than samples above 70% total motility.

Successful artificial insemination of swine is not a simple process and many factors are involved in achieving adequate fertility rates. The following conclusions were drawn from the data:

Farrowing rates of artificially inseminated swine approach those of naturally mated swine when A.I. is closely supervised. Litter size is not appreciably different following artificial insemination as compared to natural mating.

Boars used for artificial insemination may differ in their conception rates. A similiar variation in fertility also occurs in boars used naturally.

Artificial insemination technicians vary considerably in their abilities. This is indicated by marked differences in farrowing rates and average litter sizes of swine they inseminate.

Boar spermatozoa can be stored at refrigerator temperatures (5° C. to 8° C.) for at least 30 hours without greatly reducing fertility.

Two breedings during estrus increases the farrowing rate in artificially inseminated swine. Artificially inseminated sows tend to have higher farrowing percentages than artificially inseminated gilts but these differences are not always significant. Artificially inseminated sows have larger litters then do similiarly mated gilts.

The backflow of semen at the time of artificial insemination does not necessarily indicate ineffective insemination technique. In some instances improved farrowing rates are associated with the occurrance of some insemination backflow.

The progressive and total motility of boar spermatozoa upon collection can be used to determine inferior semen samples. Progressive motility should be above 40% and total motility above 70% for the most satisfactory fertility results. At these higher percentages, however, increased motility is not closely related to increased fertility of a semen sample.

These data indicate that artificial insemination of swine is practical but further investigation is needed to improve conception rates. The problems of extension and storage of boar semen require further attention as do factors relating to the detection of estrus in swine and inproved techniques of insemination.

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APPENDIX TABLE A

Breeding Information on Gilts Inseminated With Stored Semen

Gilt no.	Date bred	Treatments	Age of semen	P.M.	T.M.	Condition at slaughter
5	5-1-62	Seperated by funnel	7 da.	49%	76%	Cystic,
6	5-3-62	Other buffer	7 da.	5%	75%	Normal, non preg.
1	5-3-62	CO_2 atmosphere	7 da.	39%	64%	Normal, non preg.
7	5-3-62	CO_2 atmosphere	7 da.	39%	64%	Normal, non preg.
8	5-4-62	Other buffer	8 da.	23%	42%	Normal, non preg.
10	5-8-62	Pooled	5 da.	22%	39%	Normal, non preg.
25	5-8-62	Pooled	5 da.	22%	39%	Normal, non preg.
11	5-10-62	Standard diluter	7 da.	20%	35%	Normal, non preg.
23	5-10-62	Centrifuged	7 da.	10%	40%	Normal, non preg.
12	5-11-62	Standard diluter	7 da.	12%	50%	Normal, non preg.
13	5-12-62	Standard diluter	7 da.	15%	40%	Normal, non preg.
24	5-12-62	Centrifuged	8 da.	13%	74%	Cystic
9	5-12-62	CO_2 atmosphere	7 da.	20%	51%	Cystic
3	5-15-62	Other sugar	7 da.	24%	76%	Normal, non preg.
16	5-18-62	Standard diluter	7 da.	40%	90%	Normal, non preg.
2	5-18-62	Pooled	7 da.	10%	85%	Pregnant, 5 pigs
15	5-18-62	Pooled	7 da.	10%	85%	Normal, non preg.
30	5-18-62	CO_2 atmosphere	7 da.	38%	67%	Normal, non preg.
6*	5-22-62	Standard diluter	11 da.	3%	18%	Normal, non preg.
18	5-22-62	Other buffer	8 da.	10%	60%	Normal, non preg.
17	5-22-62	Centrifuged	8 da.	45%	60%	Normal, non preg.
26	5-22-62	Seperated	8 da.	35%	70%	Normal, non preg.
8*	5-22-62	CO_2 atmosphere	11 da.	44%	78%	Normal, non preg.
19	5-23-62	Standard diluter	9 da.	0%	75%	Normal, non preg.
1*	5-23-62	Seperated by funnel	5 da.	45%	75%	Normal, non preg.
20	5-25-62	Centrifuged	5 da.	35%	50%	Normal, non preg.
7*	5-25-62	Centrifuged	5 da.	35%	50%	Pregnant, 3 pigs
21	5-25-62	Seperated by funnel	5 da.	40%	65%	Normal, non preg.
3*	5-28-62	Other buffer	5 da.	50%	92%	Normal, non preg.
10*	5-28-62	Other buffer	5 da.	50%	92%	Normal, non preg.
14	5-29-62	Standard diluter	8 da.	40%	70%	Normal, non preg.
12*	5-29-62	Other buffer	5 da.	25%	65%	Normal, non preg.

APPENDIX TABLE A (CONTINUED)

Breeding Information on Gilts Inseminated With Stored Semen

Gilt no.	Date bred	Treatments	Age of semen	P.M.	T.M.	Condition at slaughter
22	5-29-62	Other buffer	5 da.	25%	65%	Normal, non preg.
29	5-31-62	Centrifuged	7 da.	18%	52%	Normal, non preg.
4	6-24-62	Pooled	8 da.	12%	43%	Normal, non preg.
104	7-11-62	Centrifuged	7 da.	12%	50%	Pregnant, 8 pigs
108	7-10-62	Centrifuged	6 da.	25%	45%	Pregnant, 8 pigs
110	7-19-62	Centrifuged	8 da.	2%	19%	Normal, non preg.
107	7-19-62	Centrifuged	8 da.	2%	19%	Normal, non preg.
103	7-24-62	Centrifuged	8 da.	15%	37%	Normal, non preg.
102	7-24-62	Centrifuged	8 da.	15%	37%	Normal, non preg.
101	7-25-62	Centrifuged	5 da.	18%	50%	Immature
113	7-28-62	Centrifuged	8 da.	10%	40%	Cystic
106	7-29-62	Centrifuged	5 da.	25%	55%	Normal, non preg.
111	7-31-62	Centrifuged	7 da.	20%	35%	Normal, non preg.
121-7	7-24-62	Pooled	8 da.	14%	56%	Normal, non preg.
122-4	7-25-62	Pooled	8 da.	12%	46%	Normal, non preg.
18-8	7-26-62	Pooled	8 da.	25%	40%	Normal, non preg.
14-8	7-28-62	Pooled	7 da.	18%	52%	Normal, non preg.
16-9	7-29-62	Pooled	7 da.	20%	35%	Pregnant, 7 pigs
17-2	8-9-62	Pooled	9 da.	52%	83%	Normal, non preg.
45-8	8-14-62	Pooled	9 da.	30%	70%	Normal, non preg.
16-3	8-14-62	Pooled	9 da.	30%	70%	Normal, non preg.
18-8*	8-15-62	Pooled	10 da.	33%	76%	Normal, non preg.
122-4*	8-16-62	Pooled	9 da.	20%	55%	Normal, non preg.

* Gilts reserviced.

ROOM USE ONLY